GENERAL LIBRARY
JUL 3 1918
UNIV. OF MICH.
SCIENCE LIBRARY

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXIV JUNE, 1918 No. 6

CONTENTS

PAGE

SHULL, A. FRANKLIN — *Effect of Environment upon Inherited Characters of Hydatina senta* 335

WARREN, DON C. — *The Effect of Selection upon the Sex-Ratio in Drosophila ampelophila* 351

GOLDFARB, A. J. — *Effects of Aging upon Germ Cells and upon Early Development. Part II.* 372

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

Jacques Loeb—*The Rockefeller Institute for Medical Research*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XXXIV. *June, 1918.* *No. 6.*

BIOLOGICAL BULLETIN

EFFECT OF ENVIRONMENT UPON INHERITED CHARACTERS OF *HYDATINA SENTA*.

A. FRANKLIN SHULL,

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF MICHIGAN.

INTRODUCTION.

Several years ago it was discovered (Shull, 1915) that two distinct parthenogenetic lines of the rotifer *Hydatina senta*, one from England, the other from Nebraska, differed in certain physiological (and perhaps structural) characters: (1) the English line laid smaller eggs than the Nebraska line; (2) the English line habitually laid a large percentage of its eggs attached to the surface film of the water, while the Nebraska line laid most of its eggs at the bottom or sides of the vessel; (3) the eggs of the English line required longer to develop than did those of the Nebraska line; (4) and when the rotifers were killed in Bouin's fluid the foot was seldom, or only slightly, retracted in the English line, but considerably retracted in the Nebraska line.

When crosses were effected between these two lines, the F_1 lines and F_2 lines were all indistinguishable from the English line in all the above-named characteristics. It seemed as if segregation and recombination had failed, and that in some way the four characters were rigidly associated one with another.

At first it was regarded as possible that the four characters were not really distinct, but were different manifestations of a single (physiological) character. That character might have been a greater permeability of the cells in one line than in the other. Thus, if the Nebraska line were more permeable to oxygen, the increased metabolism might make its eggs larger. For the same reason the eggs of the Nebraska line might develop in less time. In like manner it might be that the Nebraska rotifers, able to get the required amount of oxygen at the bottom

of the dish where the oxygen in solution was less abundant, lived most of the time at the bottom and laid their eggs there; whereas the less permeable English rotifers were forced to swim to the surface where dissolved oxygen was presumably more abundant, and laid eggs at the surface film. And if the foot muscles of the Nebraska line were more permeable to the killing fluid than were those of the English line, the greater contraction of the muscles of the former might thereby be explained.

It was possible to test the correctness of the above assumptions, in part, by artifically altering the expression of the inherited characters through changes in the environment. A number of experiments were performed to this end. However, before they were completed, an F_3 generation was obtained in which the association of the four inherited characters was broken. In this and the succeeding generations each one of the four characters was separated at least once from the others with which it was associated in the original lines.

Thus was proven that the four characters were not merely different expressions of one character. The experiments designed to test their separateness or singleness were, therefore, not completed, and were not published. It has now become necessary, however, to refer to certain of the results, and they are here described in the incomplete form in which they were left. Along with them are several experiments on the viability of fertilized eggs, as affected by external conditions. These are of interest to the experimenter from a practical standpoint, and also in relation to popular ideas concerning the fertilized eggs of *Cladocera*.

Experiments.

Effect of oxygen upon the laying of eggs at surface and bottom of water.

On each of the dates named in Table I., approximately equal numbers of females of *Hydatina* were placed in two dishes. In one was placed water oxygenated by vigorously shaking it in an atmosphere having a high percentage of oxygen. The dish was then set, uncovered, under a bell jar in which was confined an atmosphere containing the same high percentage of oxygen as that with which the water was first saturated. Since the bell

jar was being used daily for other experiments, the percentage of oxygen used was not always the same. From May 27 to June 22, and on July 8 (see Table I.), the atmosphere contained 40 per cent. of oxygen; on all other dates 60 per cent.

In the other of the two dishes was placed untreated water. This dish was also set under a jar sealed at the edges, to prevent excess of evaporation, but in ordinary air.

After 20 to 40 hours the dishes were removed and the number of eggs at the surface film and at the bottom counted. As shown in Table I., the eggs are much less abundant at the surface film in the presence of much oxygen than in air.

TABLE I.

Showing the Number of Eggs Laid at the Surface Film and at the Bottom of the Dish by Rotifers Placed in Air and in an Atmosphere Containing an Excess of Oxygen.

Date.	Air.		Excess of Oxygen.	
	Number of Eggs at Surface.	Number of Eggs at Bottom.	Number of Eggs at Surface.	Number of Eggs at Bottom.
May 27	12	5	1	15
29	37	48	16	55
June 3	22	22	16	27
22	2	42	0	25
28	62	20	19	49
29	8	25	7	29
30	43	85	18	121
July 1	38	19	17	27
2	40	33	34	39
3	45	6	14	41
4	42	36	23	76
6	12	5	8	22
8	23	10	12	22
Total	386	356	185	548
Percentage at surface	52.0		25.2	

Effect of oxygen upon the size of parthenogenetic eggs.

Eggs laid by rotifers in oxygenated water, and in untreated water, were obtained in the following manner. Three or four young females, due to begin egg laying in 6 to 12 hours, were placed in each of two watch glasses. One lot was immersed in water saturated with an atmosphere of which 40 per cent. or 60 per cent. was oxygen, and then set under a bell jar in a similar atmosphere. The other lot was placed in spring water, and the dish was set under a closed bell jar in air. After 24 hours the

watch glasses were removed, and all the eggs that were in a horizontal position were measured by means of a screw micrometer eye-piece. The measurements are given in units of the scale. They are directly comparable with the measurement of eggs in my former paper (Shull, 1915) since all the measurements were made with the same microscope and with the same lenses.

The mean length, mean breadth, standard deviation, etc., of the two lots of eggs are given in Table II.

TABLE II.

A Comparison of the Eggs Laid by the Rotifer Hydatina Senta in Oxygenated Water and Untreated Water.

	In Oxygenated Water.	In Untreated Water.	Difference.
Number of eggs measured.....	100	100	0
Mean length of eggs.....	16.208 ± 0.028	16.098 ± 0.027	0.110 ± 0.039
Mean breadth of eggs.....	14.054 ± 0.021	14.021 ± 0.018	0.033 ± 0.028
Standard deviation of length...	0.409 ± 0.019	0.404 ± 0.019	0.005 ± 0.026
Standard deviation of breadth..	0.308 ± 0.015	0.266 ± 0.013	0.042 ± 0.019

The eggs in the oxygenated water were a trifle larger than those in the untreated water, though it can hardly be said that the difference is statistically significant. However, a difference that cannot be proven by statistical treatment to be significant is not necessarily insignificant. It seems to me not improbable that the difference in length is significant, but it is very small in comparison with the difference between English and Nebraska eggs described in my earlier publication (*op. cit.*).

Effect of oxygen upon the time of development of parthenogenetic eggs.

A number of egg-laying females were put into each of two dishes in the evening. In one dish was placed ordinary water, in the other water that had been oxygenated in the manner described in the preceding experiments. The females were removed after about an hour, but the eggs which they had laid were left in the dishes. The oxygenated water in one dish was then removed and replaced with fresh oxygenated water, and the dish was set under a bell jar in an atmosphere containing an excess of oxygen (40 or 60 per cent.). To insure that mechanical disturbance or

accumulation of metabolic products did not affect the time of development of the eggs unequally, the water in the control dish was also drawn off after the rotifers were removed and replaced with fresh water. This dish was then set under a bell jar in air.

The next morning the two dishes were examined at intervals of 20 to 30 minutes, and the young rotifers removed and counted as they hatched. In this way the approximate time of development of the eggs was determined. In Table III. these times are collected in groups, to the nearest half hour. When these figures are treated statistically, they compare with one another as in Table IV.

TABLE III.

Showing the Time Required for Development of Eggs in Oxygenated Water and in Untreated Water.

	Number of Eggs Hatching in (Hours).						
	11.5.	12 0.	12.5.	13.0.	13.5.	14.0.	14.5.
Oxygenated water.....	7	10	15	23	9	1	
Untreated water.....	7	8	12	15	14	4	1

TABLE IV.

Showing the Time of Development of Eggs in Oxygenated Water and in Untreated Water. Statistical Treatment of Data in Table III.

	In Oxygenated Water.	In Untreated Water.	Difference.
Mean time of development in hours.....	12.654 ± 0.104	12.803 ± 0.129	0.149 ± 0.165
Standard deviation of time of development.....	1.239 ± 0.074	1.497 ± 0.091	0.258 ± 0.117

The eggs in oxygenated water hatched in a trifle shorter time, and somewhat more uniformly, though the difference in each case is so small that it may be insignificant. The greater uniformity of the time of development in oxygenated water (second line of Table IV.) is not improbably significant.

Effect of Oxygen upon the Contraction of the Foot Muscles.

Rotifers were placed in water saturated with an atmosphere containing 40 per cent. of oxygen, under a bell jar containing a similar atmosphere, and kept there 24 hours. They were then

removed and promptly killed in Bouin's fluid. Other rotifers from the same families were kept in ordinary water, under a bell jar in air, for 24 hours, then killed in Bouin's fluid.

The contraction of the foot muscles was noted in accordance with the following arbitrarily chosen degrees of contraction: (0) foot fully extended; (1) foot slightly contracted, toes bent to one side, but still visible; (2) foot considerably contracted, toes wholly concealed, but contraction limited to small region near toes; and (3) foot greatly contracted, region of contraction much greater than in preceding class. It is to be noted that this classification is not the same as that proposed for the English and Nebraska rotifers in my former paper (Shull, 1915). The descriptions there given were not applicable to the rotifers used in these experiments.

The degrees of contraction of the foot muscles of the two lots of rotifers is tabulated in Table V., and the statistical comparison of the figures in Table VI.

TABLE V.

Showing the Degree of Contraction of the Foot Muscles of Rotifers Kept in Oxygenated Water, and in Untreated Water, and Then Killed in Bouin's Fluid.

Degree of Foot Contraction.	Number of Rotifers of Given Foot Contraction.	
	In Oxygenated Water.	In Untreated Water.
0	35	21
1	97	111
2	64	64
3	2	2

TABLE VI.

Statistical Comparison of Data in Table V.

	In Oxygenated Water.	In Untreated Water.	Difference.
Mean foot contraction.....	1.16 ± 0.034	1.27 ± 0.030	0.11 ± 0.046
Standard deviation of foot contraction.....	0.64 ± 0.021	0.71 ± 0.024	0.07 ± 0.031

The statistical treatment is based on the assumption that each degree of contraction of the foot muscles differs from the degrees next to it by unity.

As shown in Table VI. there is less contraction of the foot

muscles in oxygenated water than in untreated water, but the difference is rather small compared with its probable error and in view of the crudity of the method of measurement may be without significance.

Effect of Temperature upon the Laying of Eggs at Surface and Bottom.

Equal numbers of rotifers were placed in a number of dishes of water. Some of the dishes were kept at room temperature which was fairly constant at 20° to 22° C. Other dishes were kept much cooler by setting them on a window sill outside. A thermometer was kept beside the latter dishes, and was frequently read. In Table VII., which gives the number of eggs laid at the surface film and at the bottom, the stated temperature

TABLE VII.

The Number of Eggs Laid at the Surface Film and at the Bottom at Room Temperature and at Considerably Lower Temperatures.

Date.	Room Temperature.		Low Temperature.		
	Number of Eggs at Surface.	Number of Eggs at Bottom.	Number of Eggs at Surface.	Number of Eggs at Bottom.	Temperature in Degrees C.
Nov. 28.....	44	74	1	6	8
Nov. 29.....	20	59	0	14	7
			0	8	
			0	9	
			0	9	
Dec. 3.....	8	20	0	6	4
			0	10	
			0	4	
			0	2	
			0	0	
Dec. 4.....	15	22	0	0	2
			0	1	
			0	1	
			0	0	
Dec. 7.....	74	20	0	3	3
			0	1	
			0	1	
Dec. 8.....	6	20	3	7	12
			2	19	
			0	0	4
			0	3	
Totals.....	167	215	6	104	
Percentage at surface....	43.7		5.5		

of these cool dishes is an estimated average. Because fewer eggs were to be expected at such low temperatures, several dishes were kept at low temperature for every one at room temperature.

There is no question here that the eggs are laid more largely at the surface when the temperature is high. The experiment was repeated on a small scale with the same result.

Effect of Temperature upon the Viability of the Fertilized Eggs.

Freezing.—Freezing of fertilized eggs was designed as a practical measure only, in order to induce those eggs to hatch which would not otherwise hatch. The attempt failed, however, as shown by the following tests.

In the first experiment about 244 eggs that remained unhatched after hatching had ceased for 12 days in the lot of eggs to which they belonged, were divided into two approximately equal groups. One group was frozen over night by immersing, in a closed vessel, in a brine-ice mixture and the other was kept in water at ordinary temperature. Of the frozen lot none hatched in 19 days thereafter. Of the control lot at room temperature, one hatched in 10 days after the beginning of the experiment, none thereafter. Freezing did not facilitate the hatching.

In the second experiment about 230 eggs that remained unhatched for 14 days after hatching of the eggs in the same lot had practically ceased were similarly divided into two groups, one of which was frozen over night and the other kept at room temperature. None of these eggs in either lot hatched in 19 days, after which time observation ceased.

From these experiments it appears that fertilized eggs not ordinarily capable of hatching can not be made to hatch by freezing.

Low Temperature Above Freezing.—Two lots of fertilized eggs from the same source and of approximately equal numbers were kept at different temperatures from the time they were laid until hatching was nearly complete. The eggs were laid between November 29 and December 8. One lot was kept at room temperature. The other was set outside on a window sill where daytime temperature, as shown by a thermometer placed beside

the dishes, ranged from 2° to 12° C. The air temperature was much below this, but was moderated in the location of the eggs by a steam radiator near the window sill inside. It appears that the water in which the eggs were placed never froze and hatching occurred during this period of low temperature. From December 22 to December 25 the atmospheric temperature was considerably above freezing but was much colder thereafter. On January 3 the "cold" dish was removed to room temperature, but hatching had been nearly completed before that time. Table VIII. shows the number of eggs that hatched.

TABLE VIII.

Showing the Effect of Room Temperature and Lower Temperatures Upon the Hatching of the Fertilized Eggs of Hydatina Senta.

Date of Beginning Experiment.	Warm.			Cold.		
	Number of Eggs.	Date of Hatching.	Number Hatched.	Number of Eggs.	Date of Hatching.	Number Hatched.
Nov. 29....	44	Dec. 6	1	43	Dec. 23	1
Dec. 2....	10	Dec. 7	1	10	Dec. 23	2
					Dec. 24	2
					Jan. 5	1
Dec. 8....	43	Dec. 10	2	44	Dec. 10	1
					Dec. 12	2
					Dec. 24	1
Total number hatching.....			4			10

Low temperature appears to favor the hatching of the eggs, not as an after effect, but during the period of low temperature.

Effect of Oxygen upon the Viability of the Fertilized Eggs.

Each of four lots of eggs from a single source was divided into two equal parts. One was placed in water of high oxygen content (saturated with an atmosphere of which either 40 per cent. or 60 per cent. was oxygen) and set under a bell jar enclosing an atmosphere containing the same proportion of oxygen as that with which the water was originally saturated. Atmospheres of 40 per cent. oxygen were used until July 7, 60 per cent. thereafter. This dish was removed from the bell jar daily and examined for hatching rotifers. The water was drawn off the eggs after examination and replaced with fresh oxygenated water and the dish returned to the bell jar.

The other dish was filled with untreated water which was drawn off daily and replaced with fresh water.

The hatching of the eggs is recorded in Table IX.

TABLE IX.

Showing the Effect of Oxygenation of the Water Upon the Hatching of the Fertilized Eggs of Hydatina Senta.

Experiment.	Date of Starting Experiment.	Oxygenated Water.			Untreated Water.		
		Number of Eggs.	Date.	Number Hatching.	Number of Eggs.	Date.	Number Hatching.
A.....	July 1, 1915	24	July 3	1	24	July 2	1
			7	1		7	1
			9	1		8	2
						12	1
				3			5
B.....	July 2, 1915	30	July 6	4	30	July 5	4
			10	1		8	1
			21	1		9	1
						10	1
						17	1
				6			8
C.....	July 4, 1915	79	July 5	1	78	July 8	1
			9	4		9	5
			10	5		10	4
			11	2		11	1
			12	3		12	3
						13	1
						14	1
				15			16
D.....	July 7, 1915	100	July 10	1	100	July 8	1
			11	2		9	1
			12	2		10	1
			12	5		11	3
			14	2		12	1
						13	8
						14	3
						21	1
						23	1
				12			20
Grand total.....	.....			36	.....		49

The eggs in oxygenated water show a somewhat lower viability in every case.

Effect of Drying upon the Viability of the Fertilized Eggs.

Dried for a Short Period.—A lot of cross-fertilized eggs—eggs laid by females of line B fertilized by males of line A of my former experiments (Shull, 1913)—were kept for seven weeks to allow hatching to take place. Practically all the hatching occurred in the second to fourth weeks of this period, almost none in the last three weeks. The 446 eggs (about two thirds of the original lot) which remained unhatched after seven weeks were divided into two nearly equal groups. One half was allowed to become dry December 8 and remain dry about 13 hours, after which it was remoistened. The other half was kept wet. The subsequent hatching of eggs from these two groups is recorded in Table X.

TABLE X.

Showing the Effect of Drying Eggs for a Short Period Upon the Proportion that Hatch in a Cross-Fertilized Lot of Eggs of Hydatina Senta.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Dec. 9	0		
10	0	Dec. 10	0
11	1	11	0
12	0	12	0
13	0	13	2
14	0	14	3
15	0	15	1
16	0	16	8
17	0	17	4
18	1	18	5
19	0	19	4
20	0	20	7
Total.....	2		34

Observations necessarily stopped December 20, but it seems likely that even more would have hatched among the eggs that were dried. Drying for a short period either favors hatching of eggs that would not otherwise have hatched or hastens the hatching of eggs whose hatching would otherwise have been spread over a long period.

The above experiment was repeated with a lot of eggs from the reciprocal cross of the foregoing—eggs laid by females of line A fertilized by males of line B. The original lot of eggs was kept seven weeks to allow of hatching. During the last three weeks

of that time very little hatching occurred. The 238 eggs that remained (less than half of the original lot) were divided into two equal parts, one of which was allowed to become dry and remain so for 13 hours on December 4. The subsequent hatching is recorded in Table XI. The results confirm the conclusions drawn from Table X.

TABLE XI.

Showing the Effect of Drying Eggs for a Short Period Upon the Proportion that Hatch in a Cross-Fertilized Lot of Eggs of Hydatina Senta. The Cross was the Reciprocal of that in Table X.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Dec. 5–20	0	Dec. 7–15	0
		16	1
		17	1
		18	16
		19	2
		20	1
Total.....	0		21

In another experiment of this kind inbred eggs (eggs laid by females fertilized by males of the same line) of a line in which a relatively small proportion of the eggs normally hatched were used. The eggs were kept five weeks, during the last 12 days of which time no eggs hatched. The eggs that remained unhatched were divided into two lots which, by mistake, were made unequal. One lot, consisting of about 130 eggs, was dried over night; the other of 106 eggs, was kept wet. The subsequent hatching of these eggs is recorded in Table XII.

TABLE XII.

Showing the Effect of Drying for a Short Period Upon the Hatching of Inbred Eggs of Hydatina Senta.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Feb. 25 to Mar. 8 ..	0	Feb. 24 to Mar. 4 .	0
Mar. 9.....	1	Mar. 5.....	1
Mar. 10 to 14.....	0	Mar. 6 to 14.....	0

Unlike the cross-fertilized eggs of Tables X. and XI., drying for a few hours neither increased the number of eggs that hatched nor hastened their time of hatching.

The foregoing experiment was repeated with inbred eggs of a line that regularly hatched more than half its eggs. The eggs remaining unhatched after five weeks were divided into two lots, one of which was dried over night and then remoistened. Though both were kept three weeks, not one egg in either lot hatched. Drying neither increased nor hastened the hatching.

Dried for Periods of Moderate Length.—These experiments differed from the foregoing in that all of the eggs were dried in one half of the experiment, instead of only those which failed to hatch under ordinary conditions. Inbred eggs were used, and drying occurred about the time when hatching was due to begin, that is, a week after the eggs were laid. Hatching began three or four days after the eggs were remoistened. Observations were continued for a month after remoistening. One lot of eggs was kept wet as a control, one was dried over night, one dried two weeks, and a fourth dried four weeks. The experiment was performed three times. The totals, without daily records, are given in Table XIII. The second division of this table really belongs to the preceding section of this paper, since it involves only a short period of drying, but it seems best to retain it here for the sake of comparison.

TABLE XIII.

Showing the Effect of Drying for Various Periods Upon the Viability of the Fertilized Eggs of Hydatina Senta. Inbred Eggs Were Used.

Experiment.	Eggs Kept Wet.		Dried Over Night.		Dried Two Weeks.		Dried Four Weeks.	
	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.
A	75	40	78	35	76	18	72	0
B	55	26	41	6	50	12	53	0
C	39	23	33	19	38	6	27	0
Total.....	169	89	152	60	164	36	152	0
Percentage hatching..	52.6		39.4		21.9		0.0	

There is plain indication in these results that drying, even for a short time, reduces the number of eggs that will hatch when again placed in water; and that the longer the period of desiccation, up to the limit of complete inhibition, the fewer the eggs that hatch.

Dried for a Long Period.—No experiment with control, involving a longer period of desiccation than four weeks, was performed; but that some eggs could withstand longer desiccation was shown. One lot of 344 cross-fertilized eggs and another of 100 inbred eggs, all from sources not used in the experiments described in this paper, were kept in dried condition from June 29 to March 23, or about nine months, when they were remoistened. In about three weeks thereafter three eggs of the first group and one of the second hatched.

All these eggs simply rested on the bottom of a watch glass when dried. It is not improbable that when the eggs, on drying, are supported by mud or sand they may remain desiccated longer and still hatch when remoistened. But even in mud the possible period of desiccation is not indefinitely long; for out of a lot of fertilized eggs in dried mud I have secured numerous young rotifers after three months of desiccation, but no eggs hatched from this lot after two years.

Summary and Discussion.

In former papers (Shull, 1913, 1915) the inheritance of size of parthenogenetic eggs, the time of development of parthenogenetic eggs, the place of laying parthenogenetic eggs (surface film or bottom), the viability of fertilized eggs (proportion that hatch), and the contractility of foot muscles was described. In this paper is shown to what extent these inherited characters may have been modified by such external agencies as temperature, oxygen, and desiccation.

It was found that if the water in which the rotifers live was exposed to an atmosphere containing more than the usual proportion of oxygen, a greater proportion of the eggs were laid at the bottom of the vessel. Under ordinary conditions the rotifers probably come to the surface because of the greater quantity of dissolved oxygen there. Those lines which normally lay their eggs mostly at the bottom probably either require less oxygen or get their oxygen more easily than lines which lay their eggs at the surface film. The hereditary character involved may therefore be the oxygen requirement or the permeability to oxygen.

If the rotifers were kept at a low temperature, their eggs were laid more largely at the bottom. This may be due to the greater concentration of oxygen in the water at low temperature, so that it is unnecessary to come to the surface so much; or to the low metabolism and hence low oxygen requirement of the animals; or to both of these reasons.

Excess of oxygen increased the size of the parthenogenetic eggs only very slightly, or not at all. Excess of oxygen decreased the time of egg development only very slightly, or not at all. It may have made the time of development a trifle more uniform, though this is hardly proven.

Rotifers kept in oxygenated water showed, when killed in Bouin's fluid, a trifle less contraction of the foot muscles than did other rotifers, though the difference was too small to constitute a proof of the action of oxygen.

Since the place of laying the eggs (surface or bottom) was so much more greatly affected by oxygen than were the other three characters tested, it would seem rather improbable even if the genetic results (Shull, 1915) had permitted such an assumption, that the several characters in which the English and Nebraska lines differ as described in the introduction to this paper, were really but a single character with several manifestations. The results described in this paper, therefore, harmonize with the genetic results previously obtained.

Freezing fertilized eggs that had remained unhatched for five to seven weeks did not induce any of them to hatch later. However, when eggs were kept from the time of laying at a low and variable temperature above freezing, more of them hatched than when kept at room temperature.

When fertilized eggs were kept in oxygenated water, a somewhat smaller proportion of them hatched than in untreated water.

Cross-fertilized eggs which had remained for seven weeks under ordinary conditions without hatching were dried over night and then remoistened. A considerable number of them were thereby caused to hatch. Inbred eggs, however, when tested in the same way, did not respond to drying. Even inbred eggs that were dried shortly after they were laid (not merely

those which failed to hatch otherwise) did not show any increase in the proportion of viable eggs. Indeed, drying had just the opposite effect on inbred eggs. Even when the eggs were kept dry only a few hours, the percentage of them that hatched was reduced; and the longer the eggs were kept dried the fewer of them hatched. Those that remained dry for four weeks did not hatch at all.

Other lines were not as sensitive to drying, for out of one lot of eggs that were dry for nine months, several eggs hatched when remoistened.

In view of the results of desiccation of inbred eggs, it is conceivable that the hatching of cross-fertilized eggs after drying was due merely to the hastening of the development of eggs whose hatching would otherwise have been spread over a long period. If we had for comparison only the experiments with inbred eggs which were dried immediately after laying, and those with cross-fertilized eggs that were dried after they had been allowed abundant time in which to hatch and had not done so, the conclusion just stated would seem not merely conceivable, but probable. However, since inbred eggs were also dried after their normal hatching period was past, and failed to hatch subsequently, whereas cross-fertilized eggs thus treated did hatch, I am inclined to believe that drying for a few hours actually caused some cross-fertilized eggs to hatch which would not have done so without drying. From the physiological viewpoint, such a difference between inbred eggs and cross-fertilized eggs is not at all improbable.

BIBLIOGRAPHY.

Shull, A. F.

'13 Inheritance in Hydatina senta. I. Viability of the resting egg and the sex ratio. Jour. Exp. Zoöl., Vol. 15, no. 1, July, pp. 49–89.

'15 Inheritance in Hydatina senta. II. Characters of females and their parthenogenetic eggs. Jour. Exp. Zoöl., Vol. 18, no. 1, January, pp. 145–186.

THE EFFECT OF SELECTION UPON THE SEX-RATIO IN DROSOPHILA AMPELOPHILA.

DON C. WARREN.

FROM THE ZOÖLOGICAL LABORATORY OF INDIANA UNIVERSITY.[1]

It is a well-known fact that sex-ratios in most animals approximate equality. The ratio is seldom one of exact equality, however, and the preponderance may be in favor either of the males or the females. The variation from equality is fairly constant for the species. For man the ratio has been found to be 100 females to 105 males; for the horse, 100 to 98; for the cow, 100 to 107; for the sheep, 100 to 97; for the pig, 100 to 111; for the dove, 100 to 105; and for the hen, 100 to 94. These variations are so constant that they cannot be attributed to chance and they are irregularities for which our present theories of sex determination offer no adequate explanation.

The writer has attempted to determine the sex-ratio of the fruit fly, *Drosophila ampelophila*. The determination was made from three unrelated stocks in which the best possible environmental conditions were provided. All matings were in single pairs. Over 35,000 flies were examined and the ratio was found to be 100 females to 95 males.

Moenkhaus tested the effect of selection upon the sex-ratio of *Drosophila ampelophila* and decided that the sex-ratio in this species is "amenable to selection." If it be true that the relative number of males and females in a strain can be varied by selection, the present theories of sex determination must be somewhat modified. Although this fact would not necessarily disprove the theories of sex determination, it would necessitate the assumption of an hypothesis of selective fertilization or of differential mortality or viability of the determining elements.

In view of the vital bearing of Moenkhaus's findings upon the theories of sex determination, it has been deemed worth while to repeat his work.

[1] Contribution No. 159.

Methods and Material.

The selection experiments were carried out upon three unrelated stocks. The stock for the first experiment was collected in Bloomington, Ind.; for the second, in Saratoga, Ind.; and for the third, in Warsaw, Ind. In the first two cases the experiments were started as soon as the stocks were collected from nature but the Warsaw stock was inbred in the laboratory for about six months before the experiment was started. At all times the greatest care was exercised to prevent contamination. The flies were provided with an abundance of food and care was taken to examine all the offspring of each pair. All matings were single pair matings.

Each experiment was conducted upon the offspring of a single pair of flies, that is, for the first experiment, a virgin female was selected from the Bloomington stock and mated to a brother. From among the offspring of this pair of flies, the parents of the two strains were obtained. The one strain in which there was selection for a relatively high number of females in comparison to the number of males, has been called the "high" strain and the one in which the selection was in the opposite direction, has been called the "low" strain. Before selection was started, a sufficient number of flies were examined to obtain a fair estimate of the sex-ratio of the stock under consideration. Moenkhaus's high and low strains were not offspring of a single pair of flies but were obtained by selecting from nature, pairs which had unusual ratios in the desired direction. Furthermore, he knew nothing of the original sex-ratio of his strains before selection began.

In each generation an attempt was made to examine the offspring of ten pairs in each strain. Unsuccessful matings, usually due to poor food conditions, sometimes prevented this. In each generation, the pair which gave the most extreme ratio in the desired direction was used as the parent of the succeeding generation. At times, technical difficulties prevented the most extreme pair being used and in these cases the next best was used. Pairs showing extreme ratios but producing a small number of offspring were not used. Since it was not possible to know which pair had the most desirable ratio till all of the offspring of all the pairs in a generation were examined, the technique of the

experiment was made somewhat difficult. This might have been overcome by isolating some males and females from each mating and then, after the counts for that generation were completed, mating the offspring of the pair giving the most extreme ratio. This would have prolonged the experiment, so to avoid delay, at about the eighth or ninth day after the young had begun to emerge, fifteen pairs were mated from among the offspring of each mating which was being examined. In this way, by the time one generation was finished, the next was almost ready to begin to emerge. Since ten matings in each strain were examined, this necessitated the mating of about300 pairs of flies in each generation. A control was also carried in each experiment in which a few hundred flies were examined each generation.

Preliminary Experiment.

This experiment was to test whether the age of the parents would in any way influence the relative number of males and females produced. Several matings were made and the adults

Table I.

Showing Results of Experiment to Test the Effect of Age of Parent Upon the Sex-Ratio.

Culture Number.	Original Mating 1 Day Old.		First Transfer 11 Days Old.		Second Transfer 18 Days Old.		Third Transfer 26 Days Old.		Fourth Transfer 34 Days Old.		Fifth Transfer 42 Days Old.		Sixth Transfer 50 Days Old.		Seventh Transfer 58 Days Old.		Eighth Transfer 62a Days Old.		Totals.	
	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
202A2.	56	68	112	110	105	111	51	40											324	329
203A2.	93	92	110	128	60	89	126	124	172	152	49	60							610	645
203A2.	156	162	135	150	58	60	78	89	136	123	91	93							654	677
204A1.	232	194	140	146	20	24													392	364
204A2.	223	234	155	156	157	151	115	115											650	656
204A2.	134	128	46	70	38	37													218	235
205A2.	214	202	109	114	86	104	115	110	12	12									536	542
205A2.	187	161	140	130	103	113	114	107											544	511
206A1.	68	58	80	55	75	77	78	76	94	89	72	95	126	118	45	45	10	5	648	618
206A2.	149	152	173	176	79	126	135	106	152	130	34	23							722	713
206A2.	173	164	179	207	141	134	129	162	156	159	183	203	71	91					1032	1120
207A1.	90	87	153	143	81	90	100	100											424	420
207A2.	120	118	129	123	86	83	84	69											419	393

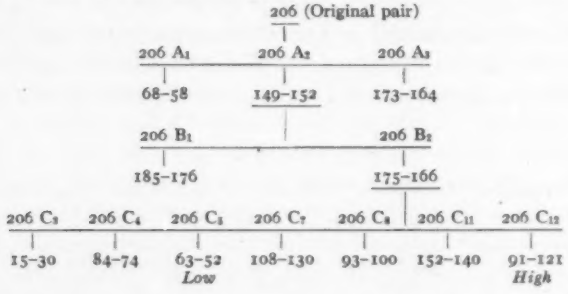
were transferred to new bottles every eight days as long as they both lived. The age of the parents does not influence the sex-ratio as is shown by the results given in Table I.

Selection Experiment I. (Bloomington Stock.)

From a stock of flies collected in Bloomington, a virgin female was selected and mated to a male from the same stock. From the offspring of this pair of flies the high and low strains were started. Before selection was begun, 2,936 flies were examined for sex. The ratio was 1,456 males to 1,480 females, or 1 male to 1.06 females. The ratio here is stated in just the reverse order from the customary form but since Moenkhaus used the reverse order, the ratios in the present discussion will be stated likewise to make the two experiments more comparable. Table II. gives the history of the origin of the high and low strains.

Table II.[1]

Showing Origin of the "High" and "Low" Strains of the Bloomington Stock.



Tables III. and IV. give the history of the high and low strains. There is no indication of the ratios having changed in the direction of the selection. In fact, in the high strain where there was selection for a relatively high number of females, the totals show a relatively lower number of females than in the low strain. The totals for the high strain were 8,837 males to 8,942 females or a ratio of 1 male to 1.012 females while in the low strain there were 8,368 males to 9,091 females or a ratio of 1 male to 1.086 females.

[1] In all tables where two numbers are expressed with a dash between them, as 68–58, the number to the left of the dash always represents the male count and the number to the right, the female count.

TABLE III.

Showing Summary of the "High" Strain of the Bloomington Stock. Experiment I.

Generation. 91 ♂-121 ♀ (*1 : 1.33*)

A (From Mating 206C11)

B	2	4	5	6	7	8	9	10	11	13	Totals.
	145–150	200–224	216–229	188–198	179–177	229–183	154–162	99–85	111–116	121–140	1642–1664
	1 : 1.03	*1 : 1.12*	*1 : 1.06*	*1 : 1.05*	*1 : .99*	*1 : .80*	*1 : 1.05*	*1 : .85*	*1 : 1.05*	*1 : 1.16*	*1 : 1.013*
C	1	3	4	5	6	8	10	12		13	
	78–65	106–118	153–148	167–179	53–53	176–165	143–136	127–115		42–69	1045–1048
	1 : .83	*1 : 1.11*	*1 : .97*	*1 : 1.07*	*1 : 1*	*1 : .94*	*1 : .95*	*1 : .91*		*1 : 1.64*	*1 : 1.003*
D	2	3	5	6	7	9	10	11	12	14	
	201–233	83–83	115–110	92–90	221–204	117–125	95–104	37–35	139–160	284–254	1384–1399
	1 : 1.16	*1 : 1.0*	*1 : .96*	*1 : .99*	*1 : .92*	*1 : 1.07*	*1 : 1.09*	*1 : .95*	*1 : 1.15*	*1 : .89*	*1 : 1.01*
E	1	2	3	4	5	6	7	10	12	13	
	117–99	184–176	223–225	194–173	302–279	179–180	252–278	93–111	195–194	36–31	1775–1746
	1 : .85	*1 : .96*	*1 : 1.01*	*1 : .89*	*1 : .92*	*1 : 1.01*	*1 : 1.1*	*1 : 1.19*	*1 : .99*	*1 : .86*	*1 : .984*
F	1	2	3	4	5	7	9	12	13	14	
	179–177	208–188	79–70	98–128	188–154	124–142	149–159	158–154	113–139	52–61	1348–1372
	1 : .988	*1 : .90*	*1 : .89*	*1 : 1.31*	*1 : .82*	*1 : 1.15*	*1 : 1.07*	*1 : .97*	*1 : 1.241*	*1 : 1.18*	*1 : 1.018*
G	1	2	5	6	8	10	11	12	14	15	
	85–96	166–168	31–55	164–174	219–223	125–135	144–143	70–80	111–119	86–96	1201–1289
	1 : 1.13	*1 : 1.01*	*1 : 1.77*	*1 : 1.06*	*1 : 1.02*	*1 : 1.08*	*1 : .99*	*1 : 1.143*	*1 : 1.07*	*1 : 1.12*	*1 : 1.073*
H	3			4		8	9	10			
	95–101			116–103		64–57	50–35	117–128			442–424
	1 : 1.06			*1 : .89*		*1 : .89*	*1 : .70*	*1 : 1.09*			*1 : .959*

Grand Total.....8837–8942
1 : 1.012

TABLE IV.

Showing Summary of the "Low" Strain of the Bloomington Stock. Experiment I.

Generation.											
A	63 ♂–52 ♀ (1 : .83) (From Mating 206C_8)										
B	1	3	4	5	7	8	9	10			Totals.
	164–228	227–248	109–113	138–146	190–187	35–88	185–184	191–197			1239–1391
	1 : 1.39	*1 : 1.09*	*1 : 1.04*	*1 : 1.06*	*1 : .98*	*1 : 2.51*	*1 : .99*	*1 : 1.03*			*1 : 1.123*
C	1	4	5	7	8	9	10	11			
	123–135	51–53	291–327	105–119	120–114	60–44	176–181	52–71			978–1044
	1 : 1.1	*1 : 1.04*	*1 : 1.12*	*1 : 1.13*	*1 : .95*	*1 : .73*	*1 : 1.03*	*1 : 1.37*			*1 : 1.067*
D	2	6	7	9	10	11	12	13	14	15	
	175–207	153–128	136–160	130–126	133–158	192–196	85–104	225–261	135–158	181–199	1545–1697
	1 : 1.18	*1 : .84*	*1 : 1.18*	*1 : .97*	*1 : 1.19*	*1 : 1.03*	*1 : 1.22*	*1 : 1.16*	*1 : 1.17*	*1 : 1.2*	*1 : 1.098*
E	1	2	4	5	8	9	10	11	12	15	
	176–141	125–123	138–131	141–135	151–151	139–142	285–277	167–141	193–234	180–180	1695–1655
	1 : .8	*1 : .98*	*1 : .95*	*1 : .96*	*1 : 1*	*1 : 1.02*	*1 : .97*	*1 : .84*	*1 : 1.21*	*1 : 1*	*1 : .976*
F	1	2	3	4	6	7	9	11	12	14	
	275–252	174–167	145–179	115–105	173–190	194–180	164–264	246–266	219–215	181–202	1886–2020
	1 : .92	*1 : .96*	*1 : 1.23*	*1 : .91*	*1 : 1.10*	*1 : .93*	*1 : 1.61*	*1 : 1.08*	*1 : .981*	*1 : 1.11*	*1 : 1.071*
G	1	6	9	11	14	15					
	114–134	84–180	89–83	205–190	37–30	84–132					613–749
	1 : 1.18	*1 : 2.14*	*1 : .93*	*1 : .93*	*1 : .81*	*1 : 1.57*					*1 : 1.221*
H	1	4	5	7	8	10	12	13	14	17	
	52–64	56–72	59–87	48–70	38–47	47–53	8–14	9–9	45–61	50–58	412–535
	1 : 1.23	*1 : 1.29*	*1 : 1.47*	*1 : 1.46*	*1 : 1.24*	*1 : 1.13*	*1 : 1.75*	*1 : 1*	*1 : 1.36*	*1 : 1.16*	*1 : 1.298*

Grand Total.....8368–9091
1 : 1.086

Selection Experiment II. (Saratoga Stock.)

In the first experiment, a much larger number of flies were examined than were examined by Moenkhaus. But since opposite results were obtained, a criticism might be offered that the writer, by chance, selected a stock which was not affected by selection. So the second experiment was made upon a new and unrelated stock. This stock was collected at Saratoga, Indiana. Fifty matings were made from among the offspring of a single pair. The extremes of these fifty were used as parents of the high and low strains. The counts of these fifty pairs are given in Table V. There were 21,019 flies examined before selection began and the stock showed a ratio of 1:1.0512, or 10, 247 males to 10,772 females.

Table V.

Showing the Origin of the "High" and "Low" Strains of the Saratoga Stock.

♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
264	**235**[1]	202	222	252	241	213	248	179	149
157	165	246	236	157	190	187	168	266	263
208	230	290	280	163	202	241	235	224	234
285	256	192	232	214	237	232	257	109	129
137	160	218	223	176	188	245	205	184	171
173	190	187	217	243	282	75	106	226	206
193	187	251	222	160	189	245	225	195	233
155	168	192	207	200	280	188	171	183	168
252	236	**181**	**307**[2]	212	275	129	175	226	222
288	271	183	187	200	225	235	247	234	220

Grand Total..... 10,247 ♂'s to 10,772 ♀'s

The results of the selection in this experiment are given in Tables VI. and VII. In this stock there were greater individual variations from the normal sex-ratios in each direction but they did not breed true. The totals show the high strain to have a higher relative number of females than the low strain. They are as follows: for the high strain, 7,377 males to 8,365 females, or a ratio of 1:1.134; for the low strain, 10,923 males to 11,246 females or a ratio of 1:1.029. Considering these totals, it might seem that something had been accomplished by selection but when the totals are considered generation by generation the results do not seem so conclusive. If selection has been the factor which

[1] From this mating originated the "low" strain.

[2] From this mating originated the "high" strain.

TABLE VI.

Showing Summary of the "Low" Strain of the Saratoga Stock. Experiment II.

Generation.											Totals.
A	264 ♂–235 ♀ (1 : .89)										
B	1	2	3	4	5	6	7	8	9	10	
	146–150	159–202	199–152	199–213	131–129	83–91	81–78	109–119	108–100	107–121	1322–1355
	1 : 1.03	*1 : 1.27*	*1 : .76*	*1 : 1.07*	*1 : .99*	*1 : 1.10*	*1 : .96*	*1 : 1.09*	*1 : .926*	*1 : 1.13*	*1 : 1.024*
C	1	2	3	4	5	6	7		8	10	
	144–161	135–143	210–208	132–130	138–149	157–138	224–251		45–44	90–84	1275–1308
	1 : 1.12	*1 : 1.06*	*1 : .99*	*1 : .98*	*1 : 1.08*	*1 : .88*	*1 : 1.12*		*1 : .98*	*1 : .93*	*1 : 1.0259*
D	1	2	3	4	5	6	7	8	9	10	
	270–294	212–242	174–135	193–217	223–211	256–246	67–81	219–215	308–308	267–263	2189–2212
	1 : 1.09	*1 : 1.14*	*1 : .78*	*1 : 1.12*	*1 : .95*	*1 : .96*	*1 : 1.21*	*1 : .98*	*1 : 1*	*1 : .985*	*1 : 1.0105*
E	1	2	3	4	5	6	7	8	9	10	
	137–136	106–115	50–65	146–169	115–130	153–153	109–138	206–188	32–39	160–149	1214–1282
	1 : .99	*1 : 1.08*	*1 : 1.3*	*1 : 1.16*	*1 : 1.13*	*1 : 1*	*1 : 1.27*	*1 : .91*	*1 : 1.22*	*1 : .931*	*1 : 1.056*
F	2		3		4		5	6			
	166–176		199–213		72–84		171–210	62–37			670–720
	1 : 1.06		*1 : 1.07*		*1 : 1.17*		*1 : 1.23*	*1 : .60*			*1 : 1.0746*
G	1	2	3	4	5	6	7	8	9	10	
	43–41	101–97	130–137	124–121	79–90	154–170	90–103	148–156	68–67	205–213	1142–1195
	1 : .95	*1 : .96*	*1 : 1.05*	*1 : .98*	*1 : 1.14*	*1 : 1.10*	*1 : 1.14*	*1 : 1.05*	*1 : .985*	*1 : 1.039*	*1 : 1.0464*
H	1	2	3	4	5	6	7	8	9	10	
	199–197	172–169	144–152	169–179	151–152	124–141	92–101	210–225	163–185	114–107	1538–1608
	1 : .99	*1 : .98*	*1 : 1.06*	*1 : 1.06*	*1 : 1.01*	*1 : 1.14*	*1 : 1.10*	*1 : 1.07*	*1 : 1.135*	*1 : .938*	*1 : 1.0455*
I	1	2	3	4	5	6	7	8	9	10	
	184–178	182–183	164–137	184–180	74–97	111–110	187–216	136–116	177–168	174–181	1573–1566
	1 : .967	*1 : 1.005*	*1 : .835*	*1 : .978*	*1 : 1.31*	*1 : .991*	*1 : 1.155*	*1 : .853*	*1 : .949*	*1 : 1.04*	*1 : .996*

Grand Total..... 10,923–11,246
1 : 1.029

TABLE VII.

Showing Summary of the "High" Strain of the Saratoga Stock. Experiment II.

Generation.											
A	181 ♂–307 ♀ (1 : 1.696)										
B	1	2	3	4	5	6	7	8	9	10	Totals.
	149–163	145–152	147–212	208–194	201–178	172–198	143–149	240–240	22–22	207–231	1634–1739
	1 : 1.09	*1 : 1.05*	*1 : 1.442*	*1 : .932*	*1 : .885*	*1 : 1.151*	*1 : 1.04*	*1 : 1*	*1 : 1*	*1 : 1.116*	*1 : 1.064*
C	2		4		6	8	9	10			
	72–81		122–109		163–158	117–118	106–148	144–136			724–750
	1 : 1.125		*1 : .893*		*1 : .969*	*1 : 1.008*	*1 : 1.306*	*1 : .944*			*1 : 1.036*
D	1	2	3	4	5	6	7	8	9	10	
	171–192	132–157	119–203	189–324	191–221	175–212	140–222	127–189	97–169	136–149	1477–2038
	1 : 1.123	*1 : 1.189*	*1 : 1.706*	*1 : 1.714*	*1 : 1.157*	*1 : 1.211*	*1 : 1.586*	*1 : 1.488*	*1 : 1.742*	*1 : 1.096*	*1 : 1.3798*
E	1	2	3	4	5	6	7	8	9	10	
	107–143	111–180	152–144	93–109	88–104	207–181	70–106	168–228	98–99	33–43	1127–1337
	1 : 1.34	*1 : 1.62*	*1 : .95*	*1 : 1.17*	*1 : 1.18*	*1 : .874*	*1 : 1.51*	*1 : 1.36*	*1 : 1.01*	*1 : 1.30*	*1 : 1.186*
F	2	3	4	5	6	7	8	9		10	
	258–276	50–37	118–107	119–102	260–221	81–104	33–28	163–206		49–56	1131–1137
	1 : 1.07	*1 : .74*	*1 : .907*	*1 : .86*	*1 : .85*	*1 : 1.284*	*1 : .85*	*1 : 1.264*		*1 : 1.14*	*1 : 1.0053*
G	1	2	3	4	5	7	8	9			
	33–84	82–111	152–138	32–41	78–94	42–38	27–30	89–91			536–627
	1 : 2.55	*1 : 1.35*	*1 : .91*	*1 : 1.28*	*1 : 1.21*	*1 : .88*	*1 : 1.11*	*1 : 1.022*			*1 : 1.1698*
H	2		3	4	5	6		9			
	74–79		154–151	108–129	40–28	99–89		37–42			512–518
	1 : 1.068		*1 : .9805*	*1 : 1.19*	*1 : .70*	*1 : .899*		*1 : 1.135*			*1 : 1.0117*
I	1		3		4	6		8			
	63–81		22–15		60–56	68–49		23–18			236–219
	1 : 1.28		*1 : .68*		*1 : .93*	*1 : .72*		*1 : .78*			*1 : .928*

Grand Total..... 7,377–8,365
1 : 1.134

has made the difference between the two strains, the difference should be most evident in the later generations of the experiment. This is not true, for a moment's calculation will show that the last three generations in each strain do not show as extreme an average ratio in the desired direction as those which preceed tehm. Although the difference between the totals of the two strains may be sufficiently large to be considered significant, it seems more probable that it is a chance variation, especially since the first experiment showed a similar variation in the direction opposite selection.

Selection Experiment III. (Warsaw Stock.)

In order to make the work still more conclusive, selection was attempted upon a third stock. This stock was collected at Warsaw, Indiana, and kept as a stock culture in the laboratory for about six months before the experiment was started.

Here forty-two pairs were mated from among the offspring of a single pair and the extremes of these matings were used as the parents of the high and low strains.[3] There were 11,190 flies examined before the selection began, 5,448 males to 5,742 females, a ratio of 1 : 1.0539. The counts of these matings are given in Table VIII.

Table VIII.

Showing Origin of the "High" and "Low" Strains of the Warsaw Stock.

♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
38	43	91	82	125	106	226	220	255	247
66	72	106	101	155	150	110	114	136	143
133	169	135	156	**235**	**238**[2]	80	72	84	77
192	208	41	39	102	105	74	93	94	100
85	75	105	128	48	61	114	129	187	200
147	**187**[1]	178	189	186	186	195	203	89	79
170	192	91	102	127	142	179	199	175	171
105	113	101	104	187	220	158	156		
125	119	106	114	112	138				

Grand Total..... 5,448 ♂'s to 5,742 ♀'s

[1] From this mating originated the "high" strain.

[2] From this mating originated the "low" strain.

[3] It will be seen in Table VIII, that the pair used as the parents of the low strain did not produce the most extreme ratio in that direction. This mistake was due to a mathematical error which was not checked up until it was too late to rectify the experiment.

In the latter part of this experiment there was a diveation from the usual methods. The element of time was sacrificed to be able to make a larger number of matings in each generation. A number of males and females were isolated from each mating, being examined, and held to be mated after all of the counts for the generation were finished. By this method it was necessary to make matings only from the cultures which had given the most desirable ratios but in so doing a considerable amount of time was lost between each generation.

This third attempt to modify, by selection, the sex-ratio did not result in bringing about any very significant difference between the two strains. (Tables IX. and X. give the results of selection upon this stock.) The ratios computed from the totals in each strain showed a slight difference in the desired direction but this was so slight that it might be due to chance. Therefore it seems that no definite conclusions can be drawn from this experiment. In the low strain the totals were 9,673 males to 9,951 females, or a ratio of 1 : 1.0287 and in the high strain 12,327 males to 12,898 females, or a ratio of 1 : 1.0463.

Conclusion.

Since three distinct and unrelated stocks of flies show no significant effect of selection, it seems safe to conclude from these data that Moenkhaus's conclusion concerning the amenability of the sex-ratio in *Drosophila ampelophila* will not hold.

Discussion of Moenkhaus's Work.

Moenkhaus concludes from his work on the effect of selection on sex-ratios in *Drosophila ampelophila* that sex-ratios are "strongly transmissible and amenable to the process of selection." From his paper it is somewhat difficult to tell whether Moenkhaus intended to say that he had developed by selection, strains characterized by high and low female ratios or that by selection, he simply maintained a high and low strain which he found in nature. His results are given in the discussion which follows.

By way of explanation it might be said that Moenkhaus called the strain in which he selected for a relatively higher female ratio the "female" strain, and the one in which he selected in

TABLE IX.

Showing Summary of the "Low" Strain of the Warsaw Stock. Experiment III.

Generation.											Totals.
A	235 ♂s–238 ♀s (*1 : 1.013*)										
B	2	3	4	5	6	7	9	10			
	183–171	266–256	141–144	150–155	112–139	227–216	224–254	155–125			1458–1460
	1 : .934	*1 : .962*	*1 : 1.02*	*1 : 1.033*	*1 : 1.24*	*1 : .951*	*1 : 1.13*	*1 : .806*			*1 : 1.0013*
C	1	2	3	4	5	6	7	8	9	10	
	222–207	234–252	231–242	105–112	211–254	164–193	247–246	158–160	96–115	111–109	1779–1890
	1 : .932	*1 : 1.077*	*1 : 1.048*	*1 : 1.067*	*1 : 1.204*	*1 : 1.177*	*1 : .996*	*1 : 1.013*	*1 : 1.198*	*1 : .982*	*1 : 1.062*
D	1	2	3	4	5	6	7	8	9	10	
	163–151	226–238	141–153	139–125	206–227	204–209	200–297	142–177	193–187	188–196	1802–1960
	1 : .926	*1 : 1.053*	*1 : 1.085*	*1 : .899*	*1 : 1.10*	*1 : 1.025*	*1 : 1.49*	*1 : 1.246*	*1 : .969*	*1 : 1.04*	*1 : 1.087*
E	1	2	3	4	5	6	7	8			
	51–46	96–111	37–45	125–119	84–89	56–48	101–101	73–68			
	1 : .902	*1 : 1.156*	*1 : 1.216*	*1 : .952*	*1 : 1.059*	*1 : .857*	*1 : 1*	*1 : .93*			
E	9	10	11	12	13	14	15				
	88–92	101–112	86–78	88–86	71–94	63–84	53–62				1173–1235
	1 : 1.045	*1 : 1.109*	*1 : .907*	*1 : .977*	*1 : 1.324*	*1 : 1.33*	*1 : 1.169*				*1 : 1.053*
F	1	3	4	5	6	7	8	9	10	11	
	151–123	100–72	192–158	135–152	113–96	121–110	117–129	41–32	97–80	99–123	
	1 : .815	*1 : .72*	*1 : .823*	*1 : 1.126*	*1 : .849*	*1 : .909*	*1 : 1.102*	*1 : .78*	*1 : .83*	*1 : 1.242*	
F	12	13	14	15	16	17	18	19	20	21	
	95–123	154–153	44–33	68–96	209–202	59–57	169–189	70–73	55–52	51–43	2140–2096
	1 : 1.295	*1 : .99*	*1 : .75*	*1 : 1.41*	*1 : .966*	*1 : .97*	*1 : 1.118*	*1 : 1.043*	*1 : .945*	*1 : .843*	*1 : .979*
G	1	2	4	7	9	10	12	13	15	16	18	19
	64–64	103–97	51–72	25–27	28–38	39–26	59–79	36–34	42–27	79–83	33–39	34–22
	1 : 1	*1 : .94*	*1 : 1.41*	*1 : 1.08*	*1 : 1.36*	*1 : .67*	*1 : 1.34*	*1 : .94*	*1 : .64*	*1 : 1.05*	*1 : 1.18*	*1 : .647*
G	20	21	22	23	24	25	26	27	28	30	31	
	19–37	55–46	29–29	88–78	34–34	89–69	104–108	75–79	65–71	115–112	55–39	1321–1310
	1 : 1.95	*1 : .84*	*1 : 1*	*1 : .89*	*1 : 1*	*1 : .78*	*1 : 1.04*	*1 : 1.05*	*1 : 1.09*	*1 : .974*	*1 : .709*	*1 : .9916*
	Grand Total.....											9673–9951
												1 : 1.0287

TABLE X.

Showing Summary of the "High" Strain of the Warsaw Stock. Experiment III.

Generation.												Totals.
A						147 ♂s–187 ♀s (*1 : 1.272*)						
B	1	2	3	4	5	6	7	8	9	10		
	126–115	195–210	52–42	53–76	119–127	138–143	191–188	194–196	206–189	208–253		1482–1539
	1 : .913	*1 : 1.08*	*1 : .81*	*1 : 1.43*	*1 : 1.07*	*1 : 1.04*	*1 : .98*	*1 : 1.01*	*1 : .92*	*1 : 1.22*		*1 : 1.039*
C	1	2	3	4	5	6	7	8	9	10		
	76–95	168–202	209–204	80–85	228–242	59–66	154–170	183–176	160–179	181–149		1498–1568
	1 : 1.25	*1 : 1.20*	*1 : .98*	*1 : 1.06*	*1 : 1.06*	*1 : 1.12*	*1 : 1.10*	*1 : .962*	*1 : 1.12*	*1 : .82*		*1 : 1.047*
D	1	2	3	4	5	6	7	8	9	10		
	43–43	69–78	79–90	141–152	149–141	147–139	194–190	195–202	193–181	146–152		1356–1368
	1 : 1	*1 : 1.13*	*1 : 1.14*	*1 : 1.08*	*1 : .946*	*1 : .946*	*1 : .979*	*1 : 1.04*	*1 : .94*	*1 : 1.04*		*1 : 1.009*
E	1	2	3	4	5	6	7	8				
	28–39	36–41	74–93	59–70	112–116	123–128	107–121	94–91				
	1 : 1.39	*1 : 1.14*	*1 : 1.26*	*1 : 1.19*	*1 : 1.04*	*1 : 1.04*	*1 : 1.13*	*1 : .97*				
E	9	10	11	12	13							
	92–105	52–62	84–82	67–121	105–126							1033–1195
	1 : 1.14	*1 : 1.19*	*1 : .976*	*1 : 1.806*	*1 : 1.2*							*1 : 1.157*
F	1	2	3	4	5	6	7	8	9	10		
	145–146	214–245	249–246	28–65	168–179	169–162	154–162	77–63	133–146	79–74		
	1 : 1.007	*1 : 1.15*	*1 : .988*	*1 : 2.32*	*1 : 1.07*	*1 : .96*	*1 : 1.05*	*1 : .82*	*1 : 1.098*	*1 : .94*		

TABLE X.—*Continued.*

	11	12	13	14	15	16	17	18	19	20	
F	100–102	214–228	195–226	182–199	177–149	208–214	194–207	121–135	148–157	93–86	
	1 : 1.02	*1 : 1.07*	*1 : 1.16*	*1 : 1.09*	*1 : .842*	*1 : 1.03*	*1 : 1.07*	*1 : 1.12*	*1 : 1.06*	*1 : .93*	
	21	22	23	24	25	26	27	28			
F	132–127	75–98	115–149	114–122	192–206	113–130	118–136	173–156			4080–4315
	1 : .96	*1 : 1.31*	*1 : 1.3*	*1 : 1.07*	*1 : 1.07*	*1 : 1.15*	*1 : 1.15*	*1 : .902*			*1 : 1.0576*
	1	2	3	4	5	6	7	9	10	11	12
G	57–69	58–56	85–91	49–56	33–27	84–72	26–46	44–49	90–94	112–122	92–83
	1 : 1.21	*1 : .97*	*1 : 1.07*	*1 : 1.14*	*1 : .82*	*1 : .86*	*1 : 1.77*	*1 : 1.11*	*1 : 1.04*	*1 : 1.09*	*1 : .90*
	13	14	15	16	17	18	19	20	21	22	23
G	65–67	46–51	128–109	73–75	53–49	73–86	41–44	62–49	34–32	68–76	63–98
	1 : 1.03	*1 : 1.11*	*1 : .85*	*1 : 1.03*	*1 : .93*	*1 : 1.18*	*1 : 1.08*	*1 : .79*	*1 : .94*	*1 : 1.12*	*1 : 1.56*
	24	25	26	27	28	29	30	31	32	33	34
G	36–27	39–60	80–69	53–33	52–62	134–108	42–35	50–48	49–39	103–113	71–87
	1 : .75	*1 : 1.54*	*1 : .86*	*1 : .62*	*1 : 1.19*	*1 : .81*	*1 : .83*	*1 : .96*	*1 : .796*	*1 : 1.097*	*1 : 1.23*
	35	36	37	38	39	40	41	42	43	44	45	
G	113–109	58–57	84–94	32–27	29–32	60–50	61–64	27–36	67–70	152–126	50–66	2878–2913
	1 : .97	*1 : .98*	*1 : 1.12*	*1 : .84*	*1 : 1.10*	*1 : .83*	*1 : 1.05*	*1 : 1.33*	*1 : 1.05*	*1 : .829*	*1 : 1.32*	*1 : 1.0122*

Grand Total.....12,327–12,898
1 : 1.0463

the opposite direction the "male" strain. This terminology will be used throughout this discussion. As the starting point of his female strain, he selected from nature a pair of flies which produced 52 males and 135 females and in his male strain he started with a pair which produced 84 males and 75 females.

Considering first the effect of selection upon his female strain we find here no conclusive evidence of any progressive change in the direction of the selection. (His data for this strain are given in Table XI.) To be sure, there is a slight increase in the relative number of females in his second and third generations over his first generation, but this fluctuation could easily be attributed to the small number of individuals examined. The greatest difficulty in assuming the effectiveness of selection here, is the fact that his last two generations are the ones in which he obtained the lowest relative number of females. Moenkhaus explains these low ratios of the last two generations in that he possibly made a poor selection in the preceding generation, but if we consider selection as having a cumulative effect (as Moenkhaus seems to consider it) it is difficult to see how one could lose by one poor selection all that he had accomplished in the previous selections. It must be admitted that by using as parents of each succeeding generation, pairs which threw the most extreme ratios in favor of the females, Moenkhaus was able to maintain a strain which on an average gave a relatively high number of females. But this was probably not due to any cumulative effect of selection but to the isolation of a peculiar type of female which will be discussed more fully later.

As to the male strain (Table XII)., Moenkhaus admits that the effect of his selection here has been slight and after examining his data carefully it is difficult to see how it could be assumed that there has been even a slight effect of selection. He started with a ratio (84 males to 75 females) which was only slightly different from normal and this slight difference was not transmitted to the first generation nor any succeeding one. So it seems that he had here a normal strain which would have given the same ratio regardless of the direction of the selection.

The female strain is the unusual one and is in need of an explanation. It throws some very exceptional ratios in favor of

TABLE XI.

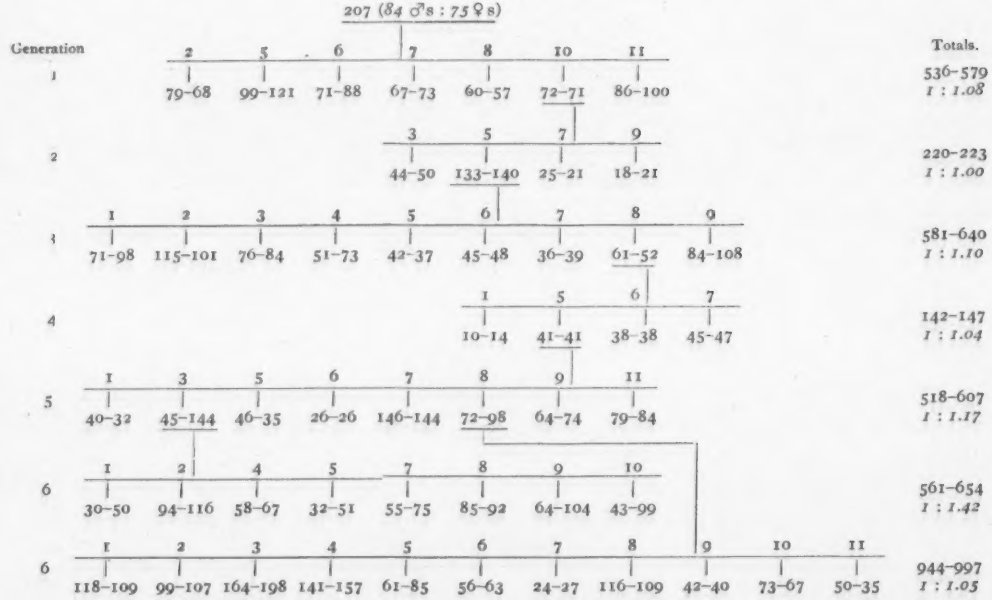
History of Moenkhaus's "Female" Strain.

206 (*52* ♂s : *135* ♀s)

Generation.	1	2	3	4	5	6	7	8	9	10	11	Totals.
1	21–37	42–68	71–164	76–71	108–125	51–80	43–67	51–46	53–103	8–56	55–102	578–969 *1 : 1.67*
2						7 21–49	8 41–94	9 55–70	10 98–178			215–391 *1 : 1.82*
3				3 15–37	4 18–54	5 25–37	7 35–71					93–201 *1 : 2.17*
4	1 26–47	2 39–56	3 33–52	5 64–67	7 53–93	8 85–162	10 54–106					354–483 *1 : 1.36*
5					4 121–139	5 32–32	6 93–101	7 40–114	8 86–132			372–518 *1 : 1.39*
5	2 51–44	3 67–53	5 41–47	6 74–78	7 120–130	10 102–136	13 41–47					496–535 *1 : 1.07*

TABLE XII.

History of Moenkhaus's "Male" Strain.



the females and these exceptional individual ratios caused the relative number of females in the female strain to be higher than normal. These unusual ratios can be readily explained by assuming that the female which Moenkhaus selected as the mother of his female strain carried a recessive sex-linked lethal factor and it will be pointed out later that all of Moenkhaus's data substantiate, very precisely, this assumption. The existence of such factors has been conclusively demonstrated by the recent work of Morgan and his students. A recessive lethal factor is any factor that brings about the death of the individual in which it occurs, provided its effect is not counteracted by the action of its normal allelomorph. Then if a lethal is sex-linked, all males which get it will die for they cannot carry its normal allelomorph since they possess but one X-chromosome. Since all males receiving the lethal factor die, this factor is never transmitted by the male and as a consequence the female can never be homozygous for it. Therefore the lethal factor has no effect on the female but she, by transmitting it to half of her sons, causes their death. Since half of the males die, a 2:1 ratio will result. A female carrying a lethal will transmit it to half of her daughters and they will always be heterozygous for it since they cannot receive it from their father.

Then the female which Moenkhaus used as the mother of his female line was probably a lethal female. It should be said that Moenkhaus is not to be criticised for not considering lethals in his paper, for lethals were not known till two years after his work was published. The original female gave a ratio which was an approximate 2:1 ratio. As mothers of the succeeding generations, Moenkhaus probably selected lethal bearing females (excluding female 5 of generation 4). These females should transmit the lethal factor to half their daughters and this expectation is realized, for from the 31 matings made in the female strain (excluding the offspring of female 5 of generation 4), fifteen 2:1 ratios resulted. Those considered 2:1 ratios are matings 3, 9 and 11 of generation 1; 7, 8 and 10 of generation 2; 3, 4 and 7 of generation 3; 1, 7, 8, and 10 of generation 4; and 7 and 8 of generation 5. So the number of 2:1 ratios obtained would justify the assumption of the presence of a lethal factor.

Another indication of the presence of a lethal factor is the fact that in generation 4, when he used female 5 which gave a normal ratio, as mother of the next generation, all of her daughters also threw normal ratios. This female did not carry the lethal factor and none of her offspring showed abnormal ratios.

A third indication of a lethal factor is the fact that Moenkhaus found in crossing his male and female strains that the female is almost wholly responsible for the transmission of the sex-ratio. For he found if females from a strain possessing a high female ratio be mated with males from a strain possessing a low female ratio or vice versa, the offspring will show a sex-ratio which is wholly or very near that of the strain from which the females were taken. If his female strain carried a lethal factor and if his male strain was a normal strain, as it appears to be, the above result would be the expected one. For, if a lethal bearing female is mated to a normal male, she will transmit the lethal factor to half her daughters but a male from the lethal strain cannot transmit the lethal factor because all of the lethal bearing males die. Therefore the end result would be as Moenkhaus found, that the offspring will show sex-ratios like that of the strains from which the females came.

Of course, the only sure way of testing for a sex-linked lethal factor is to cross the suspected female to a male carrying another sex-linked factor and the resulting F_2 will be characterized by a deficiency in the class of normal males. So it is not possible to determine beyond doubt whether the unusual ratios in Moenkhaus's female strain were due to a lethal factor. But since several lethals have already been found and all of his data substantiate this assumption, it seems that this explanation is probably the correct one.

Discussion.

The writer's three attempts to modify by selection, the sex-ratio in the fruit fly have resulted in no clear-cut evidence that it was modifiable. In each experiment, the work covered more generations and many more individuals than the work of Moenkhaus. Since Moenkhaus's results could not be obtained in any one of the three experiments, it leads one to question Moenkhaus's conclusions or, at least, to question the general application

of his findings. The fact that the difference which Moenkhaus found between his two strains can be readily explained to be due to a cause entirely independent of the cumulative effect of selection, makes his work support, rather than oppose the findings of the writer. It must be admitted that negative results in secletion work can never be conclusive, for the criticism may always be offered that selection over a greater period might have yielded different results. But, nevertheless, we feel justified in concluding from the data here presented that the sex-ratio in *Drosophila ampelophila* is not readily, if at all, modifiable by selection.

Lethals.

Since the offspring of over 700 pairs of flies were examined for sex in these experiments, and since extreme ratios were sought, it would not be surprising to find a sex-linked lethal mutation. In fact, there is fairly good evidence that one such mutation occurred. This was mating number of 6 generation G of the low strain of the Bloomington stock (Table IV.). This gave an approximate 2: 1 ratio (84 males to 180 females). Four matings were made from among the offspring of this mating and three out of the four gave 2 to 1 ratios while the fourth was doubtful. If a sex-linked lethal mutation had occurred, half of the four should have given 2 to 1 ratios and the other half normal. Since so few matings were made, it is possible that all the females chosen were lethal bearers. The stock was lost by accident at this time and further tests were not possible. But since the unusual ratio was transmitted it is probable that a mutation occurred here. In the high series of the Saratoga stock, there were several ratios which approximate a 2 to 1 ratio but none of the flies showing it transmitted this tendency to their offspring. Also in the high strain of the Warsaw stock, generation E mating 12 gave a 2 to 1 ratio. A large number of matings were made among the offspring of this pair but this unusual ratio was not transmitted to any of the progeny.

In all of the matings examined there were comparatively few ratios found which were two to one or more.[1] These extreme

[1] The unusual ratios more frequently occurred where the counts were very small. These cases were considered to have been due to some unfavorable environmental condition.

ratios were more frequently in favor of the females and in only one case was there any evidence that a sex-linked lethal mutation had occurred.

In conclusion, I wish to express my thanks to Professor Fernandus Payne for the suggestion of this problem and also for many helpful suggestions and criticism while the work was in progress. I am also greatly indebted to my wife, Elmira Shierling Warren, for help, both in the carrying out of the experiment and in the preparation of the manuscript.

SUMMARY.

1. The sex-ratio in *Drosophila ampelophila* is 100 females to 95 males.

2. The age of the parent has no effect upon the sex-ratio of its offspring.

3. The difference which Moenkhaus found between his two strains, and which he attributed to selection, was probably due to the fact that his male strain was a normal one and his female strain was a lethal bearing one.

4. The sex-ratio in *Drosophila ampelophila* is not readily, if at all, modifiable by selection.

5. There was probably one sex-linked lethal mutation in the writer's selection stocks.

BIBLIOGRAPHY.

Doncaster, L.

'14 The Determination of Sex, pp. xi + 172. Cambridge University Press, Cambridge. G. P. Putnam's Sons, New York.

Moenkhaus, W. J.

'11 The Effects of Inbreeding and Selection on Fertility, Vigor and Sex-Ratio in Drosophila ampelophila. Jour. Morph., 22: 123.

Morgan, T. H.

'12 The Explanation of a New Sex-ratio on Drosophila. Science, 36: 718.

'14a Two Sex-linked Lethal Factors in Drosophila and their Influence on the Sex-ratio. Jour. Exp. Zoöl., 17: 81.

'14b A Third Sex-linked Lethal Factor in Drosophila. Jour. Exp. Zoöl., 17: 325.

Morgan, Sturtevant, Muller, and Bridges

'15 The Mechanism of Mendelian Heredity, pp. xiii + 262. New York, Henry Holt and Co.

Rawles, Elizabeth

'13 Sex-ratios in Drosophila. BIOL. BULL., 24: 115.

Stark, A. H.

'15 The Occurrence of Lethal Factors in Inbred and Wild Stocks of Drosophila. Jour. Exp. Zoöl., 19: 531.

EFFECTS OF AGING UPON GERM CELLS AND UPON EARLY DEVELOPMENT.

PART II., CHANGES IN MODERATELY AGED EGGS AND SPERM.

A. J. GOLDFARB,

COLLEGE OF THE CITY OF NEW YORK.

CONTENTS.

PAGE.

Change in Size of Eggs 373
Change in Jelly Layer 377
Change in Membrane Formation 380
Change in Cleavage 386
(*a*) Synchronous Aging of Eggs and Sperm 386
(*b*) Fresh Sperm by Aging Eggs 389
(*c*) Aging Sperm by Fresh Eggs 395
(*d*) Longevity of Sperm and Eggs 395
(*e*) Rate of Cleavage 399
Correlation of Changes 403
Summary 408

In a previous publication (Goldfarb, '17), the writer determined the variation of freshly liberated sperm and eggs from freshly collected sea urchins of three different species. Two of these, *Toxopneustes* and *Hipponoë*, inhabit the shallow tropical waters of the Dry Tortugas, and the third, *Arbacia*, is common in the deeper colder waters near Woods Hole, Mass. In each species preliminary experiments had been made to ascertain the optimum conditions of development, such as volume and surface and filtration of sea water, concentration of eggs and sperm, etc., and such optimum conditions were used in all subsequent experimentation.

It was shown that even under these constant and optimum conditions, the freshly liberated eggs of different females varied in respect to (1) size, (2) jelly layer, (3) membrane formation, and (4) cleavage; that there was a surprisingly large variation, with respect to each of these four classes, in the eggs of different females; that the sperm of different males also varied, but to a much less degree. And finally the range of variation was determined for each species.

It was suggested that these variations served as convenient and accurate indices of the physiologic condition or vitality of the freshly removed eggs or sperm of different individuals.

In the present paper the same technique, the same three species of sea urchin, the same nomenclature and the same precautions were taken. It is proposed to determine first the physiologic condition of the germ cells, at the time of liberation, from given individuals, and then to determine the nature and the extent of the changes in the eggs and sperm as they become increasingly "overripe," "aged" or" stale."[1] In the next study, Part III., it is proposed to study other changes that appear in very late stages of overripening or ageing of the germ cells. I reserve for Part III. a discussion of the entire problem, and the bibliography.

The changes that were observed in moderately aging eggs, and those which will be discussed in detail are: (1) size, (2) jelly layer, (3) membrane formation and (4) cleavage.

I wish to acknowledge my indebtedness to Dr. A. G. Mayer, Director of the Marine Biological Laboratory of the Carnegie Institution of Washington, and to Prof. F. R. Lillie, Director of the Marine Biological Laboratory at Woods Hole, Mass., for the opportunity of working on this problem at their respective laboratories, and for the many facilities offered in connection with this work.

Effect of Age Upon Size of Eggs.

Hipponoë and Arbacia.

The diameters of freshly removed unfertilized eggs of each freshly collected female, were measured with an ocular micrometer (no. 3 eye piece and 1/6 objective) and the usual precautions concerning pressure, focusing, sampling, etc., taken. At different intervals (ages) other samples of the eggs of the same female were measured. The data are brought together in Table I.

I have shown (Goldfarb, '17) that the normal size of freshly liberated eggs of *Hipponoë* varied from 18½ to 20 ocular units in diameter, with a mode of 19½ units. In the present series

[1] By age is meant the time since removal or extrusion of ripe germ cells.

TABLE I.

SHOWS CHANGE IN SIZE AND LOSS OF JELLY LAYER WITH AGE. SIZE IS GIVEN IN OCULAR MICROMETER UNITS OF THE DIAMETER OF THE EGGS AT DIFFERENT AGES. *Hipponoë.*

No.	Date.	Age of Eggs, Hrs.	Diameter of Eggs in Ocular Micrometer Units. Number of Eggs.									Per Cent. of Eggs with Jelly Layer.	Per Cent. Loss of Jelly per Hour.
			21.0.	20.5.	20.0.	19.5.	19.0.	18.5.	18.0.	17.5.	17.0.		
1	6/15	5				*	*						
		22	*	*	*								
2	6/23	5				1	8	1				97	
		29				4	7	1				78	0.7
3	6/28	½				1	7	4				100	
		18½				8	18	3				100	0.0
4	6/28	⅔			2	5	6	2				100	
		18⅔			3	8	5	0				94	0.3
5	6/25a	2			2	7	1					100	
		5		2	8	3	1					91	
		24		7	1	3	2					40	2.7
6	6/27a	3			1	12	3					96	
		9			12	5	1					96	
		27		1	9	2	0					79	0.7
		33		0	13	3	0						
7	6/26a	0		1	3	6	3					86	
		18		3	3	6	0					77	
		24				9	5	1				62	1.0
		40						1	9	6	5	0	
		47							1	9	2	0	
8	6/26b	0			8	3						95	
		18	2	4	8	4						95	
		24			7	3						72	0.9
9	6/26c	0				0	4	8				90	
		18				0	15	2				60	
		24				3	8	3				6	3.5
		40				3	12	1				2	
		47				1	6	6				1	
10	6/25b	2				1	9	1				63	
		5				1	10	1				1	
		24					5	7	4	1		0	2.8
11	6/27b	3	2	4	3	6	0	0				80	
		9			1	4	9	1				10	
		27			1	9	2	0				1	3.3
		33			1	2	3	0	2	2	1	0	
12	6/26c	0			5	10						99	
		18		2	12	2						98	
		24		0	12	1	1					98	0.4
		40		1	11	1	1					62	
		47		1	12	1	0					17	
Variation in freshly liberated eggs of different females...			2	6	34	72	54	16	0	0	0	90	

the same variations occurred in the freshly liberated germ cells. As the eggs aged, there was a clear, considerable and progressive change in size. The observations may best be considered in three groups.

Female 1, is an example of the first group. When her eggs were 5 hours old, *i. e.*, 5 hours after liberation, their diameters were 19 and 19.5 units. When her eggs were 22 hours old, they measured 20, 20.5 and 21 units, *a clear and sharp increase in minimum, mode and maximum diameters, with age.*

In female 2, observations were made when the eggs were 5 and 29 hours old. In this female there was also an enlargement but very small. In females 3 and 4, observations were made at ½ and 18 hours respectively. In each female the eggs enlarged with age. In female 5, three observations were made, viz., 2, 5 and 24 hours, with a progressive enlargement at each successive age. In female 6, observations were made at four intervals, 3, 9, 27 and 33 hours. As in female 5, there was at first a rapid enlargement of the eggs, then a slow increase, and, in very late aging, a rapid increase again. The average diameters at each of the four intervals was 19.44, 19.80, 19.77 and 19.96.

In all the eggs of this first group, there was a cumulative increase in volume, with age, for the first 33 hours.

The second group includes such females whose eggs were observed over a longer period than 33 hours. In this group as in the first, the eggs also enlarged at first, with age, but after a longer interval there occurred a secondary diminution in size, and in very old eggs became even smaller than the norm.

For example, the eggs of female 7 were measured at 0, 18, 24, 40 and 47 hours. When 18 hours old the eggs were slightly larger than at the first observation. At 24 hours, the eggs were clearly smaller, and at 40 hours smaller still. At 47 hours they were much below their original size and below the norm for the species. The maximum size had diminished from 20.5 to 18, the mode from 19.5 to 17.5, the minimum from 19 to 17.

In female 8, there was the same initial increase and subsequent diminution towards the norm, when 24 hours old. In female 9, observations were made at 0, 18, 24, 40 and 47 hours. There occurred the usual enlargement until the 40th hour, after which there was a definite diminution in volume.

In a third group, of a few females only, the eggs though freshly removed, were in poor physiologic condition. In this group *the initial enlargement did not take place.* In these eggs

there occurred a direct diminution with age. In this group belong females 10 and 11, and probably female 7.

It should be recalled that the physiologic condition of any set of eggs was not determined merely by egg size, but by the results of several tests, such as the jelly layer test, the membrane test as well as the cleavage test, etc. By all of these tests, it was clearly and definitely shown *that eggs in good physiologic condition at the time of liberation, increased steadily with age, within the limits shown in Table I., and subsequently diminished in size by a process of fragmentation described in Part III. Eggs in physiologically*

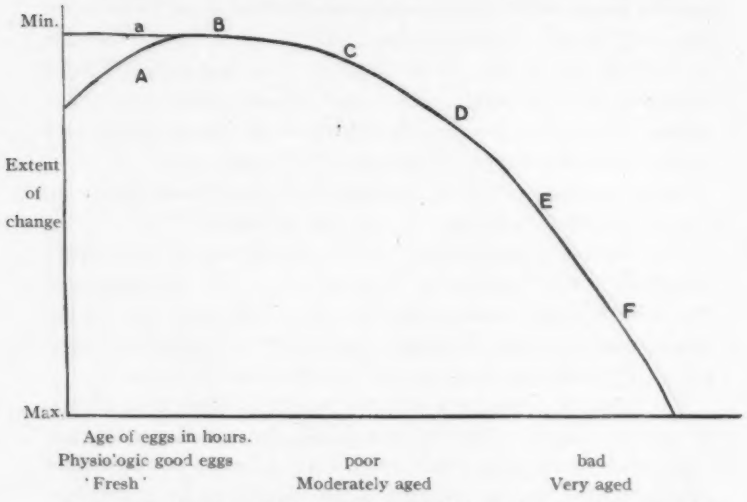


FIG. 1. Extent of morphologic and physiologic change in aging or deteriorating eggs. *A', B, C, D, E, F,* represents the change in volume, rate of membrane formation, rate of cleavage, total cleavage (*Hipponoë*). *A, B, C, D, E, F,* represents the change in width of fertilization membrane, loss of jelly, total cleavage, etc. *A', B* is period of superripening; *B, C, D,* the period of overripening or deterioration; *D, E, F,* the period of extreme deterioration or dying of the eggs.

poor condition at the time of liberation behaved like half-aged eggs, that is, they either enlarged but little, or did not enlarge at all, and subsequently diminished to and below the norm of the species.[1]

Figure 1 represents graphically and schematically the change

[1] So constant is the relation between volume and physiologic condition, tha one can predict the one from the other, with remarkable exactness.

in volume with age. Physiologically good eggs show an initial rapid increment, region *A* of the curve; then a progressively slower increment, region *B;* thirdly a progressive *diminution*, region *C*, towards *D* or even below the norm of the species to *E*. Eggs in moderately poor physiologic condition at liberation have already undergone physiologic deterioration, corresponding with part *A* within the body of the mother. Upon liberation they show only parts *B*, *C*, *D*, etc., of the curve. Eggs in very poor condition upon liberation have undergone changes represented by parts *A* and *B*, within the body of the mother, and upon liberation show only parts *C* and *D*, etc.

The significance of this increment with age lies in the changed permeability of the cortical layer which is denoted by increase in volume. This progressive increase in permeability permits increasing volumes of sea water to penetrate the egg and thus enlarge it. This process continues until partial cytolysis (fragmentation) reduces the size once again towards the norm, or until complete cytolysis destroyed the eggs altogether.

The change in size with age really measures the change in permeability of the cortical layer.

Similar observations were made upon *Arbacia* eggs, and the changes are so much in agreement with those of *Hipponoë* that it seems unnecessary to describe them here. There is the same initial increment followed by the secondary diminution with age. And the rate of change depended upon the physiologic condition of the eggs at the time of liberation and upon temperature. The two species differ in the markedly slower rate of change of *Arbacia*, due in largest part to the lower temperature of the sea water.

Effect of Age Upon the Jelly Layer.

Hipponoë and Arbacia.

The jelly layer of the same sea-urchin eggs were made clearly visible by adding Chinese black to the sea water, as suggested by F. R. Lillie. Samples of 150 to 200 consecutive freshly removed (and unfertilized) eggs of each female were examined, avoiding mechanical or chemical removal of the jelly, and the presence or absence of the jelly layer recorded. At successive intervals

other samples of the same female were examined, and the results reduced to percentages as recorded in Table I.

I have shown (Goldfarb, '17) that the freshly liberated eggs of different females vary considerably in the per cent. possessing the jelly layer. In *Hipponoë* the per cents vary from 100 to 63 per cent., in *Arbacia* from 100 to 59 per cent. These differences, I suggested, indicated corresponding differences in physiologic condition of the eggs at the time of liberation.

Whatever the condition of the eggs at the time of liberation, with increasing age there was a continuous decrease in the number of eggs with the jelly layer present.

The data may best be considered in two groups, according to the physiologic condition of the eggs at the time of the first observation.

In the first group are those females whose eggs were in good physiologic condition. This was indicated by the high per cent. (*i. e.*, above 95 per cent. with jelly layers) at the first observation, as well as by other tests. In this group are placed females 2, 3, 4, 5, 6, 8 and 12, and the corresponding percents of jelly layers was 97, 100, 100, 100, 96, 95 and 99 respectively.

In the second group are placed those females whose eggs were in poor physiologic condition. In this group are included females 7, 9, 10 and 11, whose corresponding jelly counts were, 86, 90, 63 and 80 per cent., respectively.

In both groups the surrounding jelly layer progressively disappeared with age, as seen in Table I. But the rate of loss was different. An example of the first group is female 8. When 0 hours old, 95 per cent. possessed the jelly layer, when 18 hours old 95 per cent., and when 24 hours old only 72 per cent. retained the layer. Female 11 is an example of the second group in which the loss was decidedly greater. The eggs when 3 hours old showed 80 per cent. with jelly layers, when 9 hours old but 10 per cent., when 27 hours old only 1 per cent., and when 33 hours old none of the eggs possessed the jelly envelope.

The two groups differ then, not only in original per cent. with jelly layers, but also in the rate of loss of this layer. In the first, containing physiologically good eggs, the loss of the jelly layer per hour, for the indicated intervals for the different females, was 0.7, 0.0,

0.3, 2.7, 0.7, 0.9, 0.4 per cent., with an average of 0.81 per cent. loss per hour. In the second group with physiologically poor eggs the loss was, 1.0, 3.5, 2.8, 3.3 per cent. per hour with an average of 2.65 per cent. or over three times as rapid. I cannot account for the only apparent exception, namely female 5, whose rate of loss was 2.7 per cent.

Fig. 1 represents the loss of jelly with age. With physiologically good eggs, the numbers with jelly is a maximum or nearly so, and the rate of loss with age is small, part *a* and *B*. As the eggs become physiologically poor, and in eggs which were in poor physiologic condition at the time of liberation, the numbers with jelly is much less, and the rate of loss with age is more rapid, parts *C*, *D*, etc. It should be noted in passing that parts *B*, *C*, *D* and *E*, are parallel with the corresponding parts of the curve showing change in the volume of eggs with age.

The observations upon *Arbacia* eggs are in entire accord with those of *Hipponoë*. They differ only in the slower rate of loss. One example may illustrate this slower rate. The eggs when freshly removed showed 90 per cent. with their jelly layers. When 41 hours old, 50 per cent. possessed the layer, when 46 hours, 19 per cent., when 70 hours, 1 per cent., and when 72 hours none of the eggs possessed the jelly layer. *In both species of eggs the loss of jelly was a function of age, and physiologic condition at time of the first observation.* If the loss of jelly is known, one can predict (provided proper precautions are taken) the physiologic condition of the eggs and vice versa, from the physiologic condition one may determine the loss of jelly.

Loeb, Harvey and F. R. Lillie in particular, as well as others, have recorded the loss of jelly in old eggs. My observations show not only a loss with age, which is different for different species, but I have determined the rate of loss with age. More than this, I have shown that the rate of loss depends not only upon the species and temperature, but upon the condition of the eggs at liberation, and finally that loss of jelly serves as another symptom of the physiologic condition or vitality of the eggs at any time, and that this vitality diminished at a known rate with age.

Effect of Age upon Membrane Formation.

A third sympton of aging and physiologic deterioration is the change in fertilization membrane. A number of investigators have observed that old eggs do not form fertilization membranes (Hertwig, R. and O., '86, Loeb, '03, '15, Harvey, '10, '14, F. R. Lillie, '14, etc.). Harvey ascertained the exact age in *Arbacia* beyond which, namely 52 hours, no membranes were formed. Just, '15, observed that the jelly layer, which follows fertilization in *Nereis* was retarded with aging.

Before considering in detail the changes in the fertilization membrane, with age, and the causes of such changes, it should be recalled that freshly liberated eggs of freshly collected females, when fertilized under constant and optimum conditions, varied considerably in the rate of membrane formation. Such freshly liberated eggs may be placed into three groups according to their physiologic condition, namely (1) those that form membranes within 2 minutes after fertilization, which are in good physiologic condition. (2) Those that form membranes in 3 to 7 minutes, and which are in moderately poor condition, and (3) those that do not form membranes, which are in poor physiologic condition. It should also be recalled that the rate of membrane formation is further complicated by the differential effect of eggs and sperm. I will mention but two of the experiments, which are typical, to illustrate this differential effect. In experiment 1, Table II., *the eggs of one female were fertilized by the sperm of 5 different males.* All the germ cells were 43 minutes old. Four of the males induced membrane formation in 1½ minutes, the fifth in 2 minutes. Even greater uniformity occurred when the germ cells were 1 hour old, for *the eggs of all 5 samples of the same female formed their membranes at the same time, namely 1 minute.* When the germ cells were 1½ and 2¼ hours old, the eggs again reacted in the same manner for none formed membranes.

In contrast to this uniformity of behavior, when the eggs of one female were fertilized by different males, are those experiments in which the eggs of different females were fertilized by the one male. For example, in experiment 2, the eggs of 3 females were

fertilized by the one male, when the germ cells were 20 minutes old. Fertilization membranes first appeared in the three groups of eggs as follows, 3½, 1½ and 1¾ minutes respectively. When the germ cells were 80 minutes old, they appeared in 2⅔, 1⅔, 1⅚ minutes; when 140 minutes old, 1⅔, 1½ and 1 minute; when 280 minutes, ⅔, 1¼ and 1 minute.

In the other experiments similar results were obtained, i. e., uniform or nearly uniform rate of membane formation, when the eggs of one female were fertilized by different sperm, and frequently,

TABLE II.*a*.

SHOWS CHANGE IN RATE OF MEMBRANE FORMATION WITH AGE, OF EGGS OF DIFFERENT FEMALES. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Minutes.	Minutes Required Before first Membranes Appeared.						Average No. Minutes.
			Male 1.	2.	3.	4.	5.	6.	
1	7/3	43	2	1½	1½	1½	1½		1.6
		59	1	1	1	1	1		1.0
		89	N	N	N	N	N		N
		140	N	N	N	N	N		N
			Female 1.	2.	3.	4.	5.		
2	7/14	20	3½	1½	1¾				2.25
		80	2⅔	1⅔	1⅚				2.05
		140	1⅔	1⅔	1				1.39
		210	1¼	1¼	1¼				1.25
		280	⅔	1¼	1				0.97
		370–600	N	N	N				N
3	7/21	120	1½	1					1.25
		323	1⅓	1					1.15
		450	N	N					N
4	7/16	160	N	N	2½	2½	0	2½	2.5
		300	N	N	N	N	N	1½	0.5
		500–600	N	N	N	N	N	N	N
5	7/7	75	2	3	2½	3½			2.75
		84	1	2	1½	2			1.62
		180	2	2	4½	6			3.62
6	7/12	17	1⅔	1	1				1.22
		42	1½	1	1				1.16
		99	½	¾	¾				0.66
		161	⅓	1	⅔				0.66
		214	⅓	⅔	¾				0.53
		294	2½	⅚	N				1.11
		360	5½	N	N				1.83
		420	N	N	N				N
7	7/19	130	3	3	2	1½	1½	1½	2.08
		240	N	2	2	1⅓	1⅓	1⅓	1.36
		350	N	2	2	2	2	2	1.83
		470	N	3½	4	N	2½	2½	2.08

N = no membrane in 10 minutes.

wide differences when the eggs of different females were fertilized by the one male. There was a far greater variation among the eggs of different females than among the sperm of different males, or, putting the matter in a slightly different way, the extent of variation in development is conditioned much more by the eggs than by the sperm.

With these facts in mind, we may proceed with the examination of the effect of age upon the membrane formation in physiologically "good," "moderately poor" and "poor" eggs. The data are brought together in Tables II.*a* and II.*b*.

TABLE II.*b*.

SHOWS CHANGE IN RATE OF MEMBRANE FORMATION OF AGEING EGGS AND SPERM.

Arbacia.

No.	Date.	Age of Germ Cells in Hours.		Minutes Required Before Membranes Appeared.						Average No. Minutes.
		♀.	♂.	Female 1.	2.	3.	4.	5.	6.	
1	8/12	4	4	2	1½	3	2	2		2.1
		23	23	3	7	N	N	N		5.0
		28–48	28–48	N	N	N	N	N		N
2	8/14	4	4	2	2	2	2	2	2	2.0
		24	1	N	N	3½	2	N	N	2.7
		31	8	N	N	N	N	N	N	N
		49	1¼	N	N	N	N	N	N	N
		5	5	1½	1½	½	½	½		
3	8/16	1½	1½	1½	1½	1½	1½	1½	1½	1.5
		18½	18½	3½	N	3½	3½	3½	N	3.5
		23	23	N	N	N	N	N	N	N
		24	4	2½	N	2½	N	6	N	3.6
		42	1	N	N	N	N	N	N	N
		48–75	19–¾	N	N	N	N	N	N	N
4	8/18	1	23	7	3	3	3			
5	8/19	1	45½	2	N	2½				
6	8/20	5	73	2½	4	4½	5			
		10	95	2½	N	N	N			

N = no membranes in the first 10 minutes.

In the first group, *containing physiologically good eggs, the rate of membrane formation was accelerated with age.* For example in experiment 2, the eggs of the 3 females were fertilized by one male when the germ cells were 20, 80 140, 210, 280, 370, 440 and 550 minutes old. The eggs of female number 1, for instance, formed their membranes at the above ages in 3½, 2⅔, 1⅔, 1¼ and ⅔ minutes, and none after 370 minutes. The eggs of female number 3 formed their membranes in 1¾, 1⅚, 1,

1¼ and 1 minute and none after 370 minutes. The average for all 3 females at the give ages was 2.25, 2.05, 1.39, 1.25, 0.97 minutes, and none between 370 and 600 minutes when the experiment terminated. A corresponding acceleration in the rate of membrane formation, with age, occurred in all the other experiments with physiologically good eggs.

In the second group of physiologically poor eggs, there was also an acceleration in rate of membrane formation with age, but subsequent to this, there was a period of retardation, and ultimate inability to form membranes.

For example in Experiment 5, the eggs of 4 females were fertilized by the same male when the germ cells were 75, 84 and 180 minutes old. The eggs of female no. 1 formed their membranes at these ages in 2, 1 and 2 minutes respectively, in female 3, in 2½, 1½ and 4½ minutes, in female 4 in 3½, 2 and 6 minutes respectively. Female 2 alone did not show any retardation. The average for all 4 females was *2.75, 1.62 and 3.62 minutes respectively.*

In experiment 6, of the three females whose eggs showed an initial acceleration, only one showed a secondary retardation. The observations in this experiment were made at more intermediate ages namely, 17, 42, 99, 161, 214, 294, 360, 420 and 430 minutes respectively. The eggs of female no. 1 formed membranes in 1 ⅔, 1½, ½, ⅓, ⅙ minutes and formed no more membranes after 420 minutes. The average rate for all three females for the corresponding ages, was *1.22, 1.16, 0.66, 0.53, 1.11 and 1.83 minutes* respectively, and none after 420 minutes.

In experiment 7, 5 out of 6 females showed the initial acceleration with age, and the secondary retardation. The average for the 6 females for the following ages 130, 240, 350 and 470 minutes, was *2.08, 1.36, 1.83 and 2.08 minutes respectively.*

The results may be graphically represented as in Fig. 1. The ordinates represent the inverse time or rate of membrane formation. The abcissas represent age of germ cells. With physiologically good eggs, the time and rate of membrane formation decreases with age, parts *A* and *B* of the curve. Beyond certain limits when the eggs are in poor physiologic condition, the time and rate is correspondingly increased, parts *C*, *D*, *E*,

etc. In eggs, physiologically poor at the time of liberation, there is either a short period of acceleration, part *B*, followed by a long period of retardation, parts *C*, *D*, *E*, etc. In eggs in still poorer condition, the retardation takes place from the very beginning of the experiment, parts *C*, *D*, *E*, etc. Hence the better the condition of the eggs the longer the membrane-forming period and the longer the period of acceleration. The poorer the condition the shorter the periods.

I have chosen the reciprocal curve, for in this form it becomes quite clear that it is identical with Fig. 1, showing increase in size of eggs, and decreased per cent. of jelly layers, with age. I will discuss this correlation later.

There is one other matter that deserves brief mention. It was observed repeatedly that physiologically good eggs form membranes a considerable distance from the egg surface. With increasing age and with change in rate of membrane formation, the membranes are formed closer and closer to the egg, until they could barely be distinguished from the plasma membrane, and finally did not appear at all. With this reduction in width of the perivitelline space there appeared to be a greater tendency toward scalloping or blistering in the early appearance of the membrane. The Hertwigs in '86 observed a similar narrow perivitelline space and scalloping in ageing eggs.

Arbacia.

Some observations upon *Arbacia* eggs gave similar results, with one exception, namely, there was no initial acceleration of rate in membrane formation so characteristic of *Toxopneustes*. But this lack of acceleration may however have been due to insufficient early observations. The facts are brought together in Table II.*b*.

In experiment 1 (Table II.*b*), the eggs of 2 out of the 5 females that formed membranes at all, after the first observations, showed retardation in rate with age.

In experiment 3, the eggs of 4 out of the 7 females that formed membranes after the first observation, also showed retarded rate of membrane formation.

In experiment 2, one female showed retardation, another

showed no change in rate and the other 4 did not form membranes after the first observation.

At this point the question arose whether the inability to form membranes was due to the sperm or to the egg or to both. The matter was tested as follows: *after eggs and sperm of the same age no longer formed membranes*, the eggs were fertilized by fresh sperm, and vice versa, freshly liberated eggs were tested with old sperm. In the first case, *i. e.*, stale eggs fertilized by fresh sperm, fertilization membranes reappeared, and were formed in most instances as rapidly as in previous matings. For example, in experiment 3, membranes ceased to appear when both germ cells were 23 hours old. When 24 hours old, the eggs were fertilized by 4-hour-old sperm, and membranes appeared in 3 out of 7 females. The eggs of 2 females formed membranes in 2½ minutes each, which was more rapid than when the eggs were 18½ hours old. The third formed membranes in 6 minutes. These observations showed that the first inability to form membranes was not due to the absence of membrane-forming substance in the eggs.

When fresh sperm was used with eggs 42, 48, 65 and 70 hours old, no membranes were formed. This showed that at this age the lack of membranes was now due to a change in the eggs which began about 24 hours after liberation in some females, and in 42 hours in other females. The membrane substance was gone.

In the reverse experiments, when increasingly old sperm was used to fertilize fresh eggs, membranes were formed as rapidly and as extended from the surface of the egg as when fresh sperm was used (within certain limitations). For example in experiment 4, 23-hour-old sperm caused membranes to appear in 7, 3, 3, 3 minutes in the 4 females. In experiment 5 the sperm which was 45½ hours old formed membranes in 2 of the 3 females in 2 and 2½ minutes respectively. In experiment 6, when sperm was 73 hours old, membranes appeared in 2½, 4, 4½ and 5 minutes respectively. Even when sperm was 95 hours old (experiment 6) one out of 4 females formed membranes in 2½ minutes.

These and other facts clearly show that *the rate and charac-*

ter of membrane formation, in aging eggs, are functions largely of the egg and determined by their physiologic condition at liberation and by their subsequent age. Physiologically good eggs form clear membranes rapidly with either fresh or old sperm (the maximum age of such sperm was not ascertained). Physiologically poor eggs, form membranes more slowly, closer to the egg surface or do not form membranes at all with either fresh or stale sperm. The change in rate of membrane formation with age, constitutes the third index of the physiologic deterioration of the eggs with age.

Effect of Age Upon Cleavage.

Synchronous Aging of Germ Cells.

Aging or physiologic deterioration may be measured either (1) by a change in size, or (2) a loss of the jelly layer, or (3) a change in rate and character of membrane formation. There is another, more exact and more finely graded index of the changes in the germ cell with age, namely, (4) changes in cleavage.

Hertwig, '96, and Loeb suggested that with age there was a decreasing cleavage. F. R. Lillie, '14, showed in much greater detail that there was a decrease and indicated the extent of the decrease.

In Study I., I showed that under constant and optimum conditions freshly liberated eggs from different freshly collected females frequently differed widely in the total per cent. of cleavage in a given time, even when fertilized by the same male. In *Toxopneustes* the different females vary from 11 to 87 per cent., in *Hipponoë* from 5 to 81 per cent., in *Arbacia* from 0 to 90 per cent. Such differences were ascribed to differences in physiologic condition of the eggs of the different females at the time of liberation.

In preliminary experiments, a given pair of tested sea urchins were used for successive fertilizations. From 100 to 200 of the fertilized eggs were examined at each interval, and the sperm used was freshly prepared from the dry sperm.

In one group of experiments there was an unmistakeable direct reduction in the total cleavage with age. For example, in experiment 1, the eggs of 3 females were fertilized by one male when

the germ cells were from ¼ to 6 hours old. The per cent. of the total number of eggs that cleaved at each interval was 98 per cent. when 17 minutes old, 80 per cent. when 42 minutes old, 70 per cent. when 98 minutes old, 33 per cent. when 161 minutes old, 40 per cent. when 214 minutes old, 18 per cent. when 294 minutes old, 0 per cent. when 360 minutes old. The other 2 females showed corresponding decreases. The average for the 3 females was 94, 62, 54, 28, 31, 11 and 0 per cent. respectively. See Table III.

In the other group of experiments, there was an initial increased cleavage in the eggs of some or all of the females, followed by a definite and progressive decrease. For example in experiment 6, the eggs of female 1 and 4 decreased in cleavage with age, as in the first group. But the eggs of females 2 and 3 increased in cleavage for a brief period after the first observation, and then the per cent. decreased. This appeared to me at the time to be an error in observation, but similar increments occurred in other experiments. In experiment 7 the eggs were fertilized at early and frequent intervals, namely, 7, 20, 43, 59, 89 and 140 minutes. All 5 series of mating showed an early *progressive increased cleavage*, followed after 1 hour, by a decrease. The average for all 5 series was 49, 66, 88, 62, 40 and 25 per cent.

It is probable then that in the other experiments the apparent absence of an initial increased cleavage was due to failure to make sufficiently early and sufficiently frequent intervals, or possibly that the increase in cleavage becomes evident only in eggs in poor physiologic condition.

In *Arbacia* only the direct decreased cleavage was observed. Whether an initial increase occurred I cannot say. In the first place insufficient early observations were made, and in the second place, with physiologically good eggs, the maximum or nearly maximum cleavage took place, so that any physiologic change could not be manifested by an increased cleavage.

Only two experiments are given to illustrate the behavior of aging eggs of *Arbacia*. In experiment 8, the eggs of five females fertilized by one male, averaged 80 per cent. when 4 hours old, 33 per cent. when 23 hours old, 0 per cent. when 28½ hours old. (See Table III.)

TABLE III.

SHOWS CHANGE IN TOTAL CLEAVAGE WITH PROGRESSIVE AGE OF GERM CELLS. *Toxopneustes* AND *Arbacia.*

Toxopneustes.

No.	Date.	Age of Germ Cells in Minutes.	Per Cent. of Eggs that Cleaved. Females 1.	2.	3.	4.	5.	6.	7.	Time After Fertilization, Minutes.	Average Per Cent. Cleavage.	Per Cent. Loss per Hr.
1	7/12	17	98	98	87					120	94	
		42	80	76	31						62	
		99	70	72	20						54	
		161	33	38	14						28	
		214	40	30	23						31	
		294	18	14	2						11	
		360	0	0	0						0	18.0
2	7/19	130	82[1]	96	83	96	98	94		120	91	
		240	93	99	84	89	93	91			91	
		350	92	98	82	64	81	12			71	
		470	77	64	70	4	82	10			51	7.0
3	7/16	160	50	74	81	96	18[1]	14	40	120	53	
		300	34	61	37	92	34	0	50		40	
		500	28	8	20	29	1	6	25		16	6.5
4	7/21	120	51	98						120	74	
		323	11	84							47	8.0
		450	0	0							0	
5	7/14	20	92	47	98					120	79	
		80	99	95	97						97	
		140	98	95	97						96	
		210	96	93	97						95	
		280	92	82	87						87	
		370	62	69	85						72	
		440	82	59	87						76	
		500	43	10	36						29	8.2
		810	0	0	0						0	
6	7/7	75	91	41	67	86				120	71	
		84	75	87	98	80					85	
		180	80	80	17	60					59	7.2
7	7/3	7	80	23	35	68	43			113	49	
		20	95	46	75	73	42			105	66	
		43	80	85	88	95	95			87	88	
		59	62	71	76	19	86			91	62	
		89	38	50	73	31	11			91	40	
		140	34	12	9	11	11			85	25	

Arbacia.

8	8/12	4 hrs.	95	97	18	95	95			60	80	
		23 "	57	49	0	0	60			67	33	2.5
		28½ "	0	0	0	0	0			60	0	
9	8/16	1½ "	100	98	100	88	98	92	100	64	96	
		18½ "	75	20	95	62	88	55	51	60	63	1.8
		23 "	0	0	0	0	0	0	0	60	0	

[1] Probably an error in recording.

Fig. 1 represents schematically the change in rate of cleavage with age.

The per cent. cleavage is a convenient and finely graded index not only of the physiologic condition of the eggs at the time of liberation, but at subsequent intervals. It became then a simple matter to compute the rate of deterioration with age for a given female, or group of females. The rate of deterioration, *i. e.*, the reduction in cleavage, in per cent. per hour, varied from 6.5 per cent. per hour in experiment 3, to 18.0 per cent. in experiment 1, viz: 6.5, 7.0, 7.2, 8.0, 8.2 and 18 per cent. per hour, for nearly comparable periods. These rates are strikingly greater in *Toxopneustes* (from tropical waters) than in *Arbacia* (of the temperate waters of Massachusetts). Experiments 8 and 9 are illustrative of the rate of deterioration in *Arbacia*, viz: 1.8 per cent. per hour, in experiment 9, 2.5 per cent. per hour, experiment 8. When it is recalled that the difference in temperature of the waters at the two laboratories is about 10° C., and the difference in physiologic deterioration 3 to 10 times as great, it must be evident that the difference in rate is conditioned not only by temperature but by differences in HO concentration, and the egg protoplasm of the different species.

The above data did not make clear whether the decreased cleavage was due to the deterioration of the eggs or of the sperm or both sperm and eggs. The answer to these questions could be obtained in two ways: First to fertilize increasingly old eggs each time with freshly liberated and tested sperm, which would test the rate and degree of degeneration of the eggs. Secondly to fertilize freshly liberated and physiologically good eggs with fresh suspensions of increasingly old sperm, and test the degeneration of the sperm. Both series of experiments were made.

Effect of Age Upon Eggs.

Aging Eggs fertilized by Fresh Sperm. Longevity of Eggs. Toxopneustes.

In the following experiments, eggs and sperm aged synchronously until the eggs either no longer cleaved or only a very small per cent. cleaved. Then samples of the same eggs were fertilized at each subsequent interval by freshly liberated sperm. See Table IV.

In experiment 1, for example, the eggs of 4 females fertilized

TABLE IV.

SHOWS CHANGE IN CLEAVAGE WHEN PROGRESSIVELY AGING EGGS ARE FERTILIZED BY SPERM OF VARYING AGES. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Minutes.		Per Cent. of Cleavage Eggs.						Time in Minutes.	Average Cleavage Per Cent.
		♀.	♂.	Female 1.	2.	3.	4.	5.	6.		
1	7/2	64	64	0	1	0	1			120	½
		209	19	8	85	70	10				43
		250	60	24	80	50	26				45
		309	120	3	44	14	17?				19
		400	3	8	70	2	10				22
2	7/12	420	420	6	2	0				120	3
		436	60	98	92	60					83
3	7/14	500	500	43	10	36				120	29
		600	2	50	36	81					55
		720	720	0	0	0					0
		10	600	33	0	0	10				10
4	7/19a	660	660	0	0	0	0	0	0	120	0
		660	90	95	94	13	32				59
		675	105	10	50	10	12	10	7		16
5	7/19b										
	a)	610	610	0	4					120	
				0	0						
				0	4						1
	b)	640	60	80	8						
				36	50						
				70	25						45
	c)	20	600	47	33	4	23				25
	d)	30	30	18	44	22					28
6	7/16	660	660	5	5					120	5
		7	660					91			91
		670	7	69	54	78					
				78	78						
				40							
		7	7				100				100
7	6/21	660	660	0						120	0
		660	20	18							18
		660	20	13							13
		20	660	0	0						0
		20	20	0	0						0

by the one male, gave a decreasing per cent. of cleavage with age, until the germ cells were 64 minutes old, when only 0, 1, 0 and 1 per cent. of the eggs of each of the females cleaved. The eggs were allowed to age further until they were 209 minutes old, when they were fertilized by freshly liberated sperm, and cleavage rose to 8, 85, 70 and 10 per cent. respectively. This was 42½ per cent. greater than the previous observation. It was clear that the failure to cleave when 64 minutes old was due to the almost complete deterioration of the sperm. It is not clear from this experiment how much the eggs have deteriorated. For

as I have shown the results obtained by sperm from different individuals may not be compared without further testing. In this experiment, when the eggs were 225 minutes old, and sperm only 35 minutes old, 32 per cent. cleaved. When the eggs were 250 minutes old and the sperm 60 minutes old, the average cleavage was 45 per cent. Even when the eggs were 400 minutes old, and fertilized by freshly liberated sperm, 22 per cent. cleaved. Hence it is clear that the decline to the zero mark when both germ cells were 64 minutes old, was due not to the death or incompatibility of the eggs, but of the sperm.

Even more striking results were obtained in other experiments. In experiment 2, for example, cleavage decreased progressively until the germ cells were 420 minutes old, and 6, 2 and 0 per cent. of the eggs cleaved. When the eggs 16 minutes later, *i. e.*, 7¼ hours old, were fertilized by sperm only 1 hour old, the cleavage was 98, 92 and 60 per cent. or an increase of 80 per cent. over the last reading. When the eggs of these same females were liberated, and immediately fertilized they averaged 80 per cent. hence it is probable that very little real deterioration of the eggs occurred during the first 7 hours after liberation.

In experiment 3, when the germ cells were both 8 hours old, the average cleavage was 29 per cent., when 12 hours old 0 per cent. When the eggs were 10 hours old and the sperm 2 minutes old, the average cleavage rose to 55 per cent.

In experiment 4, 11-hour-old germ cells gave 10 per cent. cleavage. Eleven-hour-old eggs by moderately fresh sperm gave 59 per cent. cleavage. Fifteen minutes later using the same eggs and sperm, only 16 per cent. cleaved. The marked reduction was due again to the rapid deterioration of the sperm of the second male.

In experiment 5, the eggs of 2 females were tested by 3 different males. When the germ cells were 5 hours old 1 per cent. cleaved. When tested by moderately fresh sperm, cleavage rose to 45 per cent.

In experiment 6, the increased cleavage with fresh sperm was 57 to 73 per cent.

In experiment 7, the small increase with fresh sperm, *i. e.*, only 18 per cent. and 13 per cent. respectively, was due to the

poor physiologic condition of the sperm as determined by tests with fresh eggs.

In every experiment, freshly liberated sperm raised the total cleavage from 0 or nearly 0 to 30, 40, 50 and even 80 per cent. of the eggs examined. There can be little doubt but that this means that the apparent death of the eggs (when no cleavage occurred) was really due to the precocious and rapid deterioration of the sperm, with moderately little deterioration of the eggs.

Hence to determine the exact physiologic condition of the eggs at any stage in the ageing cycle, and to determine the real longevity of the eggs, it is necessary to fertilize the eggs with freshly liberated sperm at each testing, as F. R. Lillie, '14, had done for a very different purpose. For example, the eggs of 4 females of experiment 1, Table V., when 1/8 hour old, gave 95

TABLE V.

SHOWS REDUCTION IN CLEAVAGE WHEN AGEING EGGS ARE FERTILIZED BY FRESH SPERM. SHOWS TRUE LONGEVITY OF EGGS. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Hours.		No. of ♂.	Female No.						Average Cleavage, Per Cent.
		♀.	♂.		1.	2.	3.	4.	5.	6.	
1	7/4	1/3	1/3	1	95						95
		20	1/2	2	50	43	95	80			67
		23	1 2/3	2	0	4	0	3			2
		24	1/10	4	0	13	0	0			3
		25	1	4	0	1	2	0			1
		46	1	5	0	2	0	0			1/2
		48	1/10	6	0	0	0	0			0
2	7/5	23	1/10	1	7	0	0				1
		25	1	2	45	0	2				—
		50	1/10	1	0	0	0				0
3	7/7	46	1/3	1	6	0	1	0			1 3/4
		46	1/4	2	0	0	1	0			1/4
		47	3/4	3	0	2	1	1			1
4	7/8	1/2	1/2	1					90	81	85
		23	1/3	1	0	22	43	25			22
		51	1/10	2	0	0	0	0			0

per cent. average cleavage; when 20 hours old, tested by fresh sperm, 67 per cent.; when 23 hours old, 7 per cent.; when 24 hours old, 3 per cent.; when 25 hours old, 1 per cent.; when 46 hours old, 1/2 per cent.; when 48 hours old, 0 per cent. These figures give a much more exact measure of the rate of deterioration of the eggs than when synchronously aged eggs and sperm

were used. There was very little deterioration or dying until the 20th hour, and then a most rapid rate of destruction until the 24th hour, and a final residual minimum extending until the 48th hour. The eggs of female 1 died at 23 hours female 4 at 24 hours, female 3 at 25 hours, female 2 at 48 hours.

Similar results were obtained in experiments 2, 3 and 4. Forty-eight hours appeared to be the maximum longevity of the eggs of *Toxopneustes* under the given experimental conditions. Table V. gives the rate of deterioration of the eggs, Table III. of the sperm.

Arbacia.

The experiments were repeated with *Arbacia* eggs, and the inquiry was pushed further. The data are brought together in Table VI. The facts may be summarized as follows:

1. Eggs which no longer cleaved when fertilized by sperm of the same age, did cleave when fertilized by freshly liberated sperm. In experiment 1, the increase was from 0 to 39 per cent.; in experiment 2, from 0 to 56 per cent.; in experiment 3, from 0 to 44 per cent., etc. This is exactly as in *Toxopneustes*.

2. With further aging there occurred a second cycle of progressively decreasing cleavage. For example in experiment 5 24-hour eggs fertilized by 1-hour sperm averaged for the 6 females 42 per cent. When the same germ cells were 31 and 8 hours respectively, they averaged only 10 per cent. A similar secondary deterioration occurred in experiment 1. The rapid deterioration was due in both groups to the more rapid destruction of the sperm than of the eggs, as in *Toxopneustes*.

3. The real deterioration of the *eggs* was determined as in *Toxopneustes* by fertilizing aging eggs with freshly liberated sperm at each testing. It was found that the eggs were very long lived. In experiment 7, 20-hour eggs showed little deterioration, 96 per cent. cleaved. Even when 41 hours, there was little deterioration, for 81 per cent. cleaved. When 48 hours 19 per cent., when 65 hours 9 per cent., when 72 hours 1 per cent. The other experiments, viz: 2, 3, 5, 6, 7, 8, 9, 10, 11, etc., gave similar results. They showed the same characteristics as *Toxopneustes* eggs, namely, *a long period of little deterioration, which in Arbacia is about 20 hour under the given experimental*

TABLE VI.

SHOWS REDUCTION IN CLEAVAGE WHEN AGING EGGS ARE FERTILIZED BY FRESH SPERM. SHOWS LONGEVITY OF EGGS OF *Arbacia*.

No.	Date.	Age of Germ Cells in Hours.		Female Number.							Time, Minutes.	Average Cleavage, Per Cent.	Loss Per Cent. per Hr.
		♀.	♂.	1.	2.	3.	4.	5.	6.	7.			
1	8/12a	4	4								60	80	
		28½	28½	0	0	0	0				60	0	
		28½	4½	29	33	28	67				60	39	
		48	½	2	½	0	0				60	1	1.8
		1½	1½								60	96	
2	8/16	23	23	0	0	0	0	0	0	0	60	0	
		24	4	81	79	82	0	65	82	8	60	56	
		42	1	30	0	80	0	0	0	0	90	15	
		42	1/6	77	0	72	0	0	0	0	60	21	
		48	1/10	90	3	79	0	0	0	0	90	24	
		65	¼	48	0	0	0	0	0	0	60	7	
		70	1½	21	0	0	0	0	0	0	60	3	1.3
		88	1½	0	0	0	0	0	0	0	60	0	
3	8/17	1½	1½	96	70	97					120	87	
		18½	18½	71	20	93						61	
		23	23	0	0	0						0	
		24	4	28	50	65						44	
		42	1	30	0	17						15	
		42	1/10	66	0	70						45	
		48	1/6	52	3	74						43	
		65	¼	45	4	1						16	
		70	1½	18	6	5						9	1.1
4	8/17	46	1½	40	41	67					60	43	
		7	25	0	0	0						0	
5	8/13	4	5	98	97	99	99	98	93		120	97	
		24	1	87	3	57	89	19	0			42	
		31	8	64	0	0	0	0	0			10	3.2
		49	1¼	0	0	0	0	0	0			0	
		49	1¼	0	0	0	0	0	0			0	
6	7/29	20	1½	73	85						120	79	
		43		34	35							34	
		51		7	5							6	2.3
7	8/1	20–22	1½	96	96						120	96	
		41		75	87							81	
		48		14	24							19	
		65		3	15							9	
		72		2									1.8
8	8/3	41	1½	11	22						120	16	
		63		8								8	
		87		0								0	
9	8/9	48	1½	9	36						120	22	
		68		0								0	
10	8/10	45	1½	2	7						120	4	
		49		1	7							4	
		51		0	0							0	
		69		0	0							0	
11	8/11	20	1½	89								89	
		40		7								7	

*Much cleavage, exact count not made.

Table VI.—*Continued.*

No.	Date	Age of Germ Cells in Hours. ♀.	Age of Germ Cells in Hours. ♂.	Female Number. 1.	2.	3.	4.	5.	6.	7.	Time, Minutes.	Average Cleavage, Per Cent.	Loss Per Cent. per Hr.
12	8/18	6	28	49	99	95	99				90	85	
		1	45½	0	0	0					60		
				91	47	97					120	81	
		5	49½	0	17	85					60		
				93	31	77					120	67	
		1/10	68	39	90	52	48				60		
				100	100	83	40				120	80	
		5	73	66	74	75	45				60		
				90	69	87	100				120	86	
		10	77	*	*	*							
		1/10	95	29	59	72	78				275	59	
13	8/12a	48	48	14							60		
				14							120	14	
	b	18	48	80							60		
				98							120	98	
	c	1/12	48	60							60		
				70							120	70	
	d	48	18	11							60		
				13							120	13	
	e	18	18	89							60		
				92							120	92	
	f	1/12	18	58							60		
				70							120	70	

conditions. Then there occurred a period of rapid deterioration, i. e., until the 40th hour, and thirdly a long period of minimum cleavage ranging from 50th to the 70th hour. Arbacia differs only in the rate of deterioration, which is several times slower than in *Toxopneustes.*

4. The longevity of *Arbacia* eggs was correspondingly longer than *Toxopneustes.* In experiment 1, the eggs of 2 out of 4 females ceased to cleave when 48 hours old. In experiment 2, the eggs of 4 females ceased to cleave at 42 hours; the fifth female at 65 hours, and the 6th female at 88 hours. In experiment 3, cleavage occurred in all 3 females at least till the 70th hour, etc., etc. The longevity ranged from 24 hours to beyond 72 hours, or about twice that of *Toxopneustes* eggs, due largely to the difference in temperature (about 10° C.) of the sea water.

Effect of Age Upon Sperm.

Freshly Liberated Eggs Fertilized by Aging Sperm. Longevity of Sperm.

In the reciprocal experiments, after the synchronously aging germ cells, no longer cleaved, the aging sperm were tested against freshly liberated eggs.

In experiment 3 (Table IV.), when the *Toxopneustes* germ cells were both 8 hours old, 29 per cent. of the eggs cleaved; when 12 hours old 0 per cent. cleaved. When the sperm were 10 hours old, they were tested by fresh eggs, 10 minutes old, and gave an average cleavage of 10 per cent. This might suggest that the rate of deterioration in synchronously aging sperm cells was conditioned by the rate of deterioration of the sperm.

In experiment 5, 10–hour–old eggs fertilized by 10-hour-old sperm averaged 1 per cent. cleavage. When the sperm was tested by fresh eggs, the cleavage rose to 25 per cent.

Similarly in experiment 6, 11-hour old germ cells averaged but 5 per cent. cleavage; the old sperm by fresh eggs gave 91 per cent. cleavage. On the other hand, in experiment 7, 11-hour-old germ cells gave 0 per cent. cleavage, in both crosses.

The explanation for these apparently contradictory data is found in a triple comparison, namely,

1. Crossing of synchronously aged germ cells.
2. Crossing of aged eggs by fresh sperm.
3. Crossing of fresh eggs by aging sperm.

If this be done it becomes apparent at once that the results are due in largest part to the condition of the egg, subject only to the ability of the sperm no matter how deteriorated to penetrate the cortical layer and initiate development.

In experiment 5, Table IV., 10-hour-old germ cells gave 1 per cent. average cleavage. When the eggs 10½ hours old were fertilized by moderately fresh sperm, 45 per cent. cleaved; the reciprocal cross, viz,, fresh eggs by the old sperm, caused only 25 per cent. to cleave. When these fresh eggs were tested by fresh sperm, 28 per cent. cleaved, *i. e., approximately the same cleavage as when fertilized by 10-hour-old sperm.* Hence low cleavage in experiment *a* was due to the sperm, while in *c* and *d* it was due to the eggs.

In experiment 6, similar results were obtained. Eleven-hour-old germ cells gave 5 per cent. cleavage. Fresh eggs used to test the old sperm gave *91* per cent. cleavage. But when these fresh eggs were tested by fresh sperm, *approximately the same percentage cleaved as when fertilized by stale sperm.*

Experiment 7 is interesting because the fresh eggs used were in

physiologically poor condition, and when fertilized by 11-hour-old sperm gave 0 per cent. cleavage. These same eggs tested by freshly liberated sperm gave the same results, namely 0 per cent.

Hence the results obtained by fertilizing fresh eggs by old sperm is determined in largest part by the physiologic condition of the eggs. The sperm plays but a minor rôle. One must be cautious, however, in any given experiment, in interpretng the results. In experiment 3, for example, the 10-hour-old sperm gave only 10 per cent. cleavage with fresh eggs. This low cleavage might have been due to the physiologically poor condition of the sperm or of the eggs. Actual tests showed that the fresh eggs were in poor condition and not the old sperm.

If the sperm deterioration is not reflected, or only to a very slight degree reflected, in a reduction in cleavage how does it happen that in all experiments *when aged eggs were fertilized by aged sperm the cleavage was far less than in either of the other two crosses.*

This result is probably due to a summation of effects, that due to the deteriorated condition of the eggs, and that due to the probable greater difficulty of aged sperm to initiate mitosis and cleavage. In the final analysis both are reducible to the condition of the egg.

Arbacia.

Further light was thrown upon these questions by the experiments with *Arbacia*.

Experiment 12, Table VI., is illustrative of the effect of aging upon the sperm. In this experiment after the germ cells no longer cleaved, the old sperm was tested against fresh eggs of 4 different females, over a long range of intervals. When the sperm were 28 hours old, the eggs of the 4 females averaged 85 per cent. cleavage (90 minutes after fertilization). When the sperm were 45½ hours old 81 per cent. of the fresh eggs of 4 other females cleaved; *when 73 hours old 86 per cent.; when 77 hours old "very many" (exact count not taken); when 95 hours old 59 per cent. of the eggs cleaved.* At this last observation the different females gave 29, 59, 72 and 78 per cent. cleavage. How much longer the sperm could have fertilized freshly liberated eggs was not determined. How much of the reduction (when the sperm

were 95 hours old) was due to a physiologic deterioration of the sperm, comparable with that in the eggs, and how much the reduction was due to an excessive mortality, as a result of which there were insufficient numbers of effective sperm, was not determined. Both factors probably play important rôles, though I am inclined to believe that a differential mortality plays a major rôle.

In the above experiment and in others, *the decrease in cleavage with these very old sperm was amazingly small, far less than in eggs of like age.* It should be noted however that the sperm in these experiments were allowed to age in the "dry" or concentrated condition, without the addition of sea water, and in this condition their metabolism is at a minimum, hence the maximum longevity.

In experiment 13, various types of crosses corroborate the above conclusions. Eggs and sperm were fertilized at varying intervals until both germ cells were 48 hours old, at which time only 14 per cent. cleaved. This 48-hour-old-sperm was tested with 18-hour-old eggs and with $\frac{1}{12}$-hour-old eggs. The 18-hour eggs gave 98 per cent. cleavage; the $\frac{1}{12}$-hour eggs 70 per cent. Samples of the same eggs were also fertilized by sperm 18 hours old. The 48-hour eggs gave 13 per cent. cleavage; the 18-hour eggs 92 per cent. and the $\frac{1}{12}$-hour eggs 70 per cent. It will be at once evident that the sperm at both ages gave a *remarkably similar cleavage per cent. with fresh, with moderately old and with old eggs, i. e.*, the sperm have undergone little if any physiologic deterioration between 18 and 48 hours after liberation. The 48-hour eggs gave 14 per cent. cleavage by 48-hour sperm and 13 per cent. with 18-hour-old sperm. The 18-hour-old eggs gave 98 per cent. cleavage with 48-hour-old sperm and 92 per cent. with 18-hour sperm. The $\frac{1}{12}$-hour-old eggs gave 70 per cent. cleavage with 48-hour-old sperm and the same per cent. with 18-hour-old sperm.

The cleavage was determined essentially or exclusively by the condition of the eggs.

The decrease in cleavage in the fresh eggs was due as in *Toxopneustes* experiment to the poorer physiologic condition of the eggs at the time of liberation. *Good fresh sperm cannot cause physiologically poor eggs to cleave to any greater extent than old*

sperm. Cleavage in physiologically good eggs is not decreased by old sperm.

From these data it will also be observed that the longevity of dry sperm was greater than the eggs in the given experimental conditions. In neither *Toxopneustes* nor in *Arbacia* was the maximum longevity of the sperm determined.

Effect of Age Upon Rate of Cleavage.

I have shown that the total number of eggs that cleaved in a given time after fertilization, decreased as the eggs aged. I propose now to show that there was, as F. R. Lillie, '14, indicated, a corresponding retardation of the rate of early cleavage, with increasing age of the egg; and in a later paper I propose to describe the irregular and abnormal character of cleavage in very aged eggs.

The rate of cleavage was ascertained by the total cleavage at three intervals, namely, (1) 40 minutes after fertilization, when the eggs first divided, (2) 1 hour after fertilization, when most and sometimes all the eggs had divided once, and (3) 2 hours after fertilization when all or very nearly all the eggs had divided at least once.

The data are brought together in Table VII.

In experiment 1, the eggs of 6 females were fertilized by one male, when the germ cells were 130, 240, 350 and 470 minutes old. A record was made at each interval, of the number of eggs that cleaved in 40, 60 and 120 minutes after each fertilization. In female no. 1, 54 per cent. cleaved within 40 minutes, when the germ cells were 130 minutes old; 37 per cent. cleaved in the same interval when 240 minutes old; 3 per cent. when 350 minutes old, 5 per cent. when 470 minutes old. *There was an undoubted decrease in the rate of first cleavage with age. The cleavage at the same ages 60 minutes after fertilization also showed a similar retardation with age of the germ cells,* for 78, 85, 51 and 59 per cent. respectively, cleaved. The total cleavage, namely 2 hours after fertilization, as I have shown before, also decreased with age. The corresponding figures were 82, 93, 92 and 77 per cent.

The average for all 6 females brings out forcibly the retardation and decrease in cleavage. The average for the germ cells 40

TABLE VII.

SHOWS CHANGE IN RATE OF CLEAVAGE WITH AGE OF GERM CELLS. THE FIGURES REPRESENT THE PER CENT. CLEAVAGE IN INDICATED TIME. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Minutes.	Female 1.			2.			3.			4.			5.			6.			Average Per Cent.			Ratio of Cleav in 40 Min.: 120 Min.	Ratio of Cleav in 60 Min.: 120 Min.
			40 Min.	60 Min.	120 Min.	40 Min.	60 Min.	120 Min.	40 Min.	60 Min.	120 Min	40 Min.	60 Min.	120 Min.	40 Min.	60 Min.	120 Min	40 Min	60 Min.	120 Min.	40 Min.	60 Min.	120 Min.		
1	7/19	130	54	78	82	70	93	96	73	88	83	82	88	96	86	94	98	91	80	94	76	86	91	83	94
		240	37	85	93	83	99	99	46	56	84	67	76	89	78	78	93	82	67	91	65	76	91	71	83
		350	3	51	92	72	94	98	22	59	82	67	57	64	70	75	89	35	26	12	44	60	71	62	84
		470	5	59	77	17	46	64	16	43	70	0	0	4	64	75	82	1	6	80	27	39	51	53	76
2	7/12	17		98			98			87															
		42	56	77	80	34	78	76	18	30	31										36	62	62	58	100
		99	0	35	70	6	50	72	1	12	20										2	32	54	3	59
		161	4	15	33	6	18	38	1	11	14										3	14	28	10	50
		214	3	24	40	6	28	30	1	11	13										3	21	31	9	67
		294	3	14	18	½	17	14	0	0	2										1	10	11	9	90
		360	0	0	0	0	0	0	0	0	0										0	0	0	0	0
		420	0	0	0	0	0	0	0	0	0										0	0	0	0	0
3	7/14	20	½	93	92	0	32	61	17	98	98										5	74	83	6	89
		80	0	87	99	0	95	95	0	35	70										0	72	88	0	81
		140	2	91	98	2	95	95	2	99	97										1	95	96	1	98
		210	8	83	96	8	63	93	12	97	97										9	81	95	9	85
		280	6	92	92	32	77	82	5	86	87										14	85	87	16	97
		370	6	61	62	4	46	69	3	47	85										4	51	65	6	78
		440	0	25	62	0	20	59	0	51	87										0	32	69	0	46
		500	0	12	43	0	½	10	0	5	36										0	6	29	0	20
4	7/16	160	0	83[1]	50	0	74	74	0	26	81	0	28	96	0	20	18				0	38	53	0	
		300	0	39	34	0	60	61	0	54	37	0	34	92	0	38	34				0	45	49	0	
		500	3		28	0		8	5		20	4		29	1		66				2		26	7	

[1] Probably an error in recording.

minutes after fertilization was *76, 65, 44 and 27 per cent.* for the 4 different ages of the germ cells. The average cleavage 60 minutes after fertilization, for the same ages, was *86, 76, 60 and 39 per cent.* and the total cleavage, *i. e.*, 2 hours after fertilization, was 91, 91, 71 and 51 per cent. respectively.

These observations suggest an explanation for the decreasing total cleavage with aging germ cells. For the decreased total may conceivably be due to a retardation of the division process, or an inhibition of this process, or both.

Records taken three hours after fertilization showed that the 2-hour observation really was the total cleavage; that very little and usually no additional cleavage occurred. *Hence the decreased total cleavage with aging germ cells was not an apparent decrease due to retardation, but a real decrease by inhibition of cleavage.*

We are concerned then with two symptoms or independent evidences of changes in the eggs caused by their aging. On the one hand, an increasing number of eggs were prevented from developing and, on the other hand, those that do develop, do so with increasing difficulty (retardation).

The relation between retardation and reduction in cleavage may be expressed as follows: When germ cells were 130 minutes old, 83 per cent. of the total number of dividing eggs cleaved within 40 minutes after fertilization; when the germ cells were 240 minutes old a less number, namely 71 per cent. of the cleaving eggs, divided in the same time. When the germ cells were 350 and 470 minutes old, 62 and 53 per cent. of the segmented eggs cleaved in 40 minutes.

In experiment 2, germ cells in poor condition were tested at 8 intervals, between ¼ hour and 7 hours, and the rate of cleavage noted at each interval. The results are essentially in accord with those of experiment 1. The per cent. cleaving in 40 minutes after each fertilization was 36, 2, 3, 3, 1, 0, 0 per cent.; for 60 minutes after fertilization, the record was 62, 32, 14, 21, 10, 0, 0; and the total cleavage 2 hours after fertilization was 62, 54, 28, 31, 11, 0, 0 respectively.

Experiment 4 is also quite in accord with the previous ones.

Experiment 3 differs from the above only in the increasing total cleavage for a brief period, followed by decreasing cleavage

with further age. The rate of cleavage follows a similar cycle; *the rate of cleavage and the total cleavage increased with the age of the germ cells until they were 280 minutes old, after which the rate was retarded and the total cleavage declined.*

Arbacia.

With *Arbacia* eggs the rate of cleavage was ascertained by noting the per cent. of the eggs that reached the 4-cell stage in 60 minutes. The data are shown in Table VIII.

TABLE VIII.

SHOWS CHANGE IN RATE OF CLEAVAGE FROM THE PER CENT. OF EGGS THAT REACH THE 4-CELL STAGE IN 60 MINUTES. *Arbacia.*

No.	Date.	Age of Germ Cells in Hours.		Female 1.	2.	3.	4.	5.	6.	7.	Time, Minutes.
		♀.	♂.								
1	8/12	4	4	24	0	0	0	3			60
		23	23	1	0	0	0	1			67
		28	4½	2	6	5	12	36			67
		24	1	1	0	0	18	1			
		30½	8	0	0	0	0	0			
2	8/16	1½	1½	80	81	90	30	80	65	93	60
		18½	18½	3	0	0	0	0	0	1	
		24	4	3	10	1	0	6	1	1	
		42	½	1	0	0	0	0	0	0	
		48	½	3	0	2	0	0	0	0	
		65	¾	4	0	0	0	0	0	0	
		70	1/6	2	0	0	0	0	0	0	
		88	1/6	0	0	0	0	0	0	0	
3	8/14b	1½	1½	42	22	67	77	90			60
		5	5	35	3	88	26	40			
4	8/14a	4	5	0	1	0	19	36			60
5	8/18	1	23	0	1	0	0				87
		5	49	0	8	0					60
		1/6	68	0	6	0					60
		5	73	5	5	3					60

In *Arbacia* as in *Toxopneustes* the total cleavage decreased and the early division process was increasingly retarded with increasing age of the germ cells. In experiment 3, for example, the eggs of 5 females were tested when their germ cells were 1½ hours old, and 59 per cent. of the eggs reached the 4-cell stage in 1 hour. When the germ cells were 5 hours old only 38 per cent. reached the 4-cell stage in the same time. In experiment 1 and 2, older eggs were used. The results were even more striking and in entire accord with the preceding experiments.

Retardation is another symptom of the deterioration not of the sperm but of the eggs.

Correlation of Changes.

I have shown firstly that the freshly liberated eggs of freshly collected females of the same species varied considerably with respect to size, jelly layer, membrane formation and cleavage; secondly the range of variability for each of these types of changes was ascertained; thirdly, these changes were correlated; fourthly, by *these correlated phenomena one could accurately and conveniently ascertain the exact physiologic condition of the eggs of any female, at any time.*

Freshly liberated eggs from freshly collected females could be readily classified into physiologically "good," "poor" and "bad" eggs, on the basis of the correlated phenomena, as follows:

	Physiologically Good Eggs.	Physiologically Poor Eggs.	Physiologically Bad Eggs.
Size:	Norm for species.	Larger than norm.	Still larger or smaller than norm.
	Small deviation from norm.	Increasing deviation.	Still further deviation.
Jelly layer:	Maximum number with jelly layers 100–90 per cent.	Increasing loss of layer 96–70 per cent.	Further loss 70–40 per cent.
Membrane formation:	Formed within 2 minutes.	Increasingly retarded. 3–7 min.	None.
Cleavage:	Maximum number cleave 100–90 per cent.	Decreasing number 90–60 per cent.	Further decrease. 60–0.

Perhaps one example chosen at random may make the matter more definite. The freshly liberated eggs of *Arbacia* (Experiment 8–12) were practically normal in size, over 90 per cent. contained the jelly layer, fertilization membranes were formed in 1½ to 2 minutes, the cleavage was 95, 97, 96 and 94 per cent. in the eggs of 4 out of 5 females. The eggs of the fifth female were larger than the norm, less than 90 per cent. possessed jelly layers, no membranes were formed upon fertilization and only 18 per cent. cleaved. The eggs of the same females were tested by a second male, and gave the same size and jelly count, as before, and membranes appeared in 2, 2, 2½, 2½ minutes respectively. The cleavage count was 72, 69, 62 and 55 per cent. This retardation

in membrane formation and reduction in cleavage was clearly due to the poor physiologic condition of the sperm. The eggs of the fifth female were likewise fertilized by this male and showed the same size and jelly count as before, but no membrane appeared and only 4 per cent. cleaved. By these tests it was definitely determined that the eggs of the first four females were in good physiologic condition, those of the fifth female in poor condition.

By these tests it is possible to state accurately whether any sample of eggs are "good," "poor" or "bad," and to state exactly to what degree of physiologic deterioration such sample of eggs may have reached. The words "good," "poor" or "bad" now have a specific meaning, in terms of definite measurable changes in size, loss of jelly, rate of membrane formation and cleavage, which changes symptomize and measure definite physiologic and morphologic changes in the eggs.

The freshly liberated eggs whose physiologic condition was determined, subsequently changed or aged or overripened, with respect to the same categories, namely, size, jelly layer, membrane and cleavage.

I have shown in this paper exactly the extent to which each of these categories varied with the aging of the germ cells. I wish now to emphasize the fact that the changes are correlated.

These correlated changes, each of which measures the degree of physiologic deterioration with age, may be briefly summarized in the following table:

	Freshly Liberated or Good Eggs.	Moderately Stale or Aged Eggs.	Very Stale or Aged Eggs.
Size:	Little deviation from the norm.	Increasing deviation in a plus direction.	Further deviation in plus or minus direction.
Jelly layer:	Maximum or close to maximum.	Decreasing.	Further decrease.
Fertilization membrane:	Within 2 minutes. Wide membranes.	Increasingly retarded. Increasingly narrow.	None.
Cleavage:	Maximum rate. Maximum number.	Increasing retardation. Decreasing numbers.	Further retardation. Further decrease.

Whatever the physiologic condition of the eggs when liberated, they undergo with age an increase in size, a decrease in the number

possessing the jelly layer, a decrease in rate of membrane formation, and a decrease in cleavage, varying in degree with the condition of the eggs at the time of liberation. And one may predict the extent of the other changes from any known one or two. If size or membrane rate are known, one may predict very approximately the other symptoms of the physiologic condition of the eggs, such as jelly and cleavage, etc.

The discussion of these results will be postponed until further data will be presented, concerning other types of changes in very aged eggs. For the present I wish to draw attention to the following considerations.

1. The aging process or processes which are symptomized in the various changes described in this paper begin not with the liberation of the eggs, but upon their maturation, within the body of the mother. This was first suggested by Loeb, and I am in entire accord with his view. Hence it follows that chronologic age (time since liberation) affords but a poor idea of the real physiologic condition of the eggs either at liberation or at any interval thereafter.

2. Aging is a continuous process, beginning within the body and continuing (with somewhat accelerated rate) outside of the body, culminating ultimately in the death of the eggs.

3. Aging was manifested in a number of ways, any one of which may serve as an index of the physiologic condition, and the degree of deterioration. *The ensemble of the various indices forms a clear and unmistakeable measure of their condition and their deterioration.*

4. While no attempt has thus far been made to describe the nature of the chemico-physical processes involved in the aging of the eggs, they may nevertheless be accurately measured. It is now possible to measure very accurately the physiologic condition of the eggs at liberation, and at any interval of time thereafter, to measure accurately the rate of deterioration or senescence under given experimental conditions. It is also possible to measure the real longevity of the eggs or of the sperm.

5. These data, and those described in Part III., afford a basis for an understanding of the nature of the aging process, and of the means of controlling senescence of the germ cells.

The discussion of the results and a more complete bibliography are given in Part III.

Summary.

The variation in size of the eggs, the per cent. with jelly layers, the rate of membrane formation and the total cleavage were ascertained for large numbers of freshly liberated eggs from freshly collected females, examined at different periods of the breeding season.

Three species of sea urchins were studied in this way, namely, *Toxopneustes* and *Hipponoë* of tropical waters, and *Arbacia* of the North Atlantic.

Large variations from the norm were observed and measured in all four categories. These variations were interpreted as indices of the physiologic condition of the eggs of each female at the time of liberation.

With the physiologic condition of freshly liberated eggs of a given female known, experiments were then instituted to ascertain the nature and extent of the changes in the germ cells, as they aged, or became overripe, under given optimum laboratory conditions.

The following is a brief statement of the changes in such aging germ cells, viz., changes in size, jelly layer, membrane formation and cleavage.

Change in Size.

Freshly liberated eggs in good physiologic condition varied but slightly from the norm. For details see text.

As these eggs aged, their volume increased continuously, until they cytolized or fragmented, and became smaller than the norm.

Freshly liberated eggs in poor physiologic condition either enlarged but little with age, or were directly reduced in size by cytolysis or fragmentation.

The nature, extent, and rate of change in size, depends upon the physiologic condition of the eggs when freshly liberated. Whatever the physiologic condition of the eggs may be, their senescence or physiologic deterioration can be very accurately measured by the degree of enlargement or reduction in their size.

Essentially the same results were obtained in the other two species.

Change in Jelly Layer.

Practically all freshly liberated eggs in good physiologic condition possess a jelly layer. Those in poor condition have a correspondingly less per cent.

With age the jelly layer was lost in an increasing number of the eggs.

The rate of loss depended upon the condition of the eggs at the time of liberation (all other conditions remaining constant).

For fresh eggs in good physiologic condition, the rate of loss per hour was 0.81 and for equally fresh eggs in poor condition 2.65 or over 3 times as rapid.

Essentially similar results were obtained in all three species.

The loss of jelly layer was a second symptom and index of the extent and the rate of ageing or senescence of the eggs.

Change in Membrane.

Freshly liberated eggs in good physiologic condition formed fertilization membranes within two minutes. The rate depended partly upon the sperm but primarily upon the physiologic condition of the eggs.

With increasing age the time required to form the fertilization membranes was at first accelerated and later retarded. In very aged eggs no membranes were formed.

Freshly liberated eggs in poor physiologic condition showed direct retardation in the rate of membrane formation.

Aging eggs which no longer formed membranes when fertilized by old sperm could be made to form membranes with fresh sperm.

The rate of membrane formation is practically independent of the sperm. It is essentially determined by the condition of the egg.

As the eggs aged the membrane appeared closer and closer to the surface of the egg; it became thinner and ultimately none was formed.

These observations are essentially the same for all three species.

The rate and character of membrane formation affords a third means of measuring senescence in eggs.

Change in Cleavage.

When freshly liberated eggs of *Hipponoë* and *Toxopneustes* in good physiologic condition were fertilized under the given optimum conditions at successive ages the total cleavage increased for a time and subsequently decreased,[1] and in extreme ageing none of the eggs segmented.

Cleavage then is an additional index of the degree of senescence or physiologic deterioration of the eggs.

There were two series of experiments, one in which the eggs and sperm aged synchronously, and the other in which they aged asynchronously. In both series the rate of decrease in cleavage with age was several times greater in *Toxopneustes* and *Hipponoë* than in *Arbacia*. This difference in rate of senescence as in rate of membrane formation, loss of jelly and change in size is due largely to differences in temperature, as well as to differences in HO concentration of the sea water, and to protoplasmic differences of the eggs.

When both germ cells aged synchronously the apparent longevity was about 11 hours for *Toxopneustes* and about 28 hours in *Arbacia*.

In asynchronous matings a more definite idea was obtained of the changes in the egg alone and in the sperm alone. When freshly liberated sperm were used to fertilize the eggs (of a female) at varying ages, with the precautions indicated in the text, the eggs showed progressively decreasing per cent. of cleavage (in *Arbacia*), but the rate of decrease was very much slower than when both germ cells aged synchronously. The decrease may be divided into three periods, the first a period of small decrease (about the first 20 hours in *Arbacia*) the second, a period of rapid and large decrease (between the 20th and 40th hour), and the third, a period of small decrease (between the 40th and 80th hour in *Arbacia*).

Eggs in poor physiologic condition at the time of liberation deteriorated at a correspondingly greater rate than physiologically good eggs.

[1] Freshly liberated eggs, of freshly collected *Arbacia*, in good physiologic condition, gave a maximum or nearly maximum cleavage and with ageing there was a direct decreasing total.

The real longevity of the eggs depended upon the physiologic condition at time of liberation, the better, the longer lived, and vice versa.

The rate of cleavage was accelerated for a time and then retarded with age, more so in physiologically poor eggs, less in good eggs.

When ageing sperm fertilized freshly liberated eggs the decrease in cleavage was surprisingly small. Very little decrease occurred when sperm were 73 hours, only a little more when sperm were 95 hours old.

The explanation for the greater longevity of the sperm is found in the fact that the sperm aged in the "dry" condition, in which they are inactive and hence minimum metabolism.

The greater decrease in cleavage in synchronously ageing germ cells is due to a summation of the injurious effects upon the sperm and upon the eggs.

In very late stages in ageing of sperm, the reduction may be due not so much to physiologic deterioration as to insufficient numbers of sperm. There is ground for belief that no matter how old or deteriorated the sperm if they are active (alive) they can penetrate the egg. It is not so clear whether such aged sperm cause parthenogenesis or sexual development.

The change in size, jelly, membrane and cleavage with aging of germ cells, are accurate, convenient and corroborative indices of chemico-physical and morphologic changes in the egg as they age, and afford convenient measures of the loss in vitality, or physical deterioration. And one change may serve for this purpose. Their ensemble is convincing.

In the next study will be considered the changes in much older germ cells leading towards their cytolysis and death.